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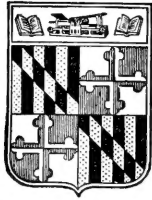
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The first volume of THE ANATOMICAL RECORD is concluded with No. 8, which was included under the cover of Vol. VII, No. 4, February 29, 1908, of this journal. In the future THE ANATOMICAL RECORD will be continued as an independent journal, published from the Wistar Institute of Anatomy. See notice of this in the pages of Vol. I, No. 8, of THE ANATOMICAL RECORD.

ON THE CEPHALIC VEINS AND SINUSES OF REPTILES,
 WITH DESCRIPTION OF A MECHANISM FOR RAISING
 THE VENOUS BLOOD-PRESSURE IN THE HEAD.

BY

HENRY L. BRUNER, PH. D.

From the Biological Laboratory of Butler College.

WITH 17 TEXT-FIGURES AND 3 PLATES.

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The cephalic veins of the Reptilia as a group have received comparatively little attention from students of vertebrate anatomy. In case of the Sauria, Ophidia, and Testudinata the larger venous trunks have been described but no attempt has been made to work out their closer relations or to follow their tributaries. A more thorough study of this field is suggested especially by the fact that the head of lizards, snakes, and turtles contains large blood sinuses whose significance has not been explained in a satisfactory way. Weber, 77, concludes, from his study of the sinus orbitalis, that this sinus acts as a substitute for the cushion of fat behind the bulbus in other vertebrates. In view, however, of the general distribution of blood sinuses throughout the head, such an explanation is obviously inadequate. It has been suggested by Hay, 92, that *Phrynosoma* utilizes a sinus for the accumulation of blood which it ejects from the orbit, but we must also account for the existence of similar sinuses in other forms which are not guilty of the blood-letting habit.

The ejection of blood by *Phrynosoma* indicates the existence of a mechanism for producing extraordinary blood-pressure in the region of

the orbit, but if such a mechanism exists, is the ejection of blood its chief function or an accessory one? If the latter is true, what is the chief function and does the mechanism occur in other forms which do not eject blood? To answer these questions in a satisfactory way it is evident that we must study not only the arrangement of blood-vessels but also their relation to the other structures of the head.

The present study is an attempt to solve these problems and others which they suggest. In the first part of the paper I shall describe the veins and sinuses of the head, particular attention being given to the Sauria. In the second part I shall show that the sinuses are always associated with a special mechanism for obstructing the vena jugularis interna and elevating the venous blood-pressure in the head. Different modifications of this mechanism will be found in the different orders of reptiles.

In regard to the functions of this mechanism, I shall show, from observations on lizards, that it plays an important part in exuviation—a process of vital importance to these reptiles. Finally, an effort will be made to trace the phylogenetic history of this moulting mechanism, to discover the conditions which determined its development and the causes of its preservation in certain groups and its disappearance in others.

In order to obtain the most satisfactory results the investigation has been confined chiefly to adult forms, in which final conditions are fully established. The anatomical descriptions are based largely on serial sections, but the results have been corrected as far as possible by means of gross dissections. The material used for the sections was injected with an aqueous solution of Berlin blue, fixed in formalin and decalcified by means of phloroglucin. The acid was neutralized on the slide by immersion in a saturated solution of ammonium chloride. The cutting was done chiefly with a large Jung microtome, and in spite of the size of some of the heads, which included *Emys* and *Phrynosoma*, excellent series were obtained.

The studies leading up to the present paper have been prosecuted for the most part in the Biological Laboratory of Butler College. A part of the sections, including *Monitor*, *Agama*, *Platydactylus*, and *Moloch*, were prepared by the author in the Anatomical Institute of Professor Wiedersheim, of the University of Freiburg, to whom he is greatly indebted for the material and for valuable suggestions in regard to literature.

PART FIRST.

THE CEPHALIC VEINS AND SINUSES OF REPTILES (SAURIA, OPHIDIA, AND TESTUDINATA).

The literature dealing with the cephalic veins of the Reptilia has been reviewed by Grosser and Brezina, 95, and does not require special consideration here. Through the investigations of these authors and the earlier work of Bojanus, 19-21, Rathke, 39, 48, and Corti, 41, it has been shown that the primitive arrangement of the cephalic veins of reptiles includes a median dorsal vein, a longitudinal vein on each side of the head, and three transverse veins which connect the dorsal and lateral veins on each side (compare Text Fig. 1). The relations of these veins may be more definitely stated as follows:

1. The median vein, *vena longitudinalis cerebri*, is an intracranial vessel which runs along the dorsal aspect of the brain, from the olfactory lobes to the foramen magnum. It lies close to the cranial wall and is enclosed in the dura mater.

2. The paired longitudinal vein, *vena jugularis interna*, is formed by the union of the orbital veins. It is an extracranial vein which runs along the side of the brain case and below the roots of the cranial nerves. In the neck the vein accompanies the vagus nerve and the carotid artery.

3. The anterior transverse vein, *vena cerebrealis anterior*, begins at the epiphysis and runs ventrad in the furrow between the forebrain and midbrain. It discharges into the vena jugularis interna a short distance behind the orbit.

4. The second transverse vein, *vena cerebrealis media*, arises from the vena longitudinalis cerebri on the dorsal aspect of the cerebellum. In the Sauria it leaves the cranium through the foramen trigemini and joins the vena jugularis interna just outside of the skull.

5. The third transverse vein, *vena cerebrealis posterior*, begins at the dorsal margin of the foramen magnum, where it is formed, along with its fellow, by the bifurcation of the vena longitudinalis cerebri. The vena cerebrealis posterior leaves the cranial cavity through the lateral part of the foramen magnum and bends directly laterad to join the vena jugularis interna.

According to Grosser and Brezina the primitive relations just described occur in earlier embryonic stages of lizards, snakes, and turtles. In the adult forms these relations are variously modified in the different orders, as shown in the more detailed accounts which follow. I begin with the Sauria, which have been more thoroughly studied than the other groups.

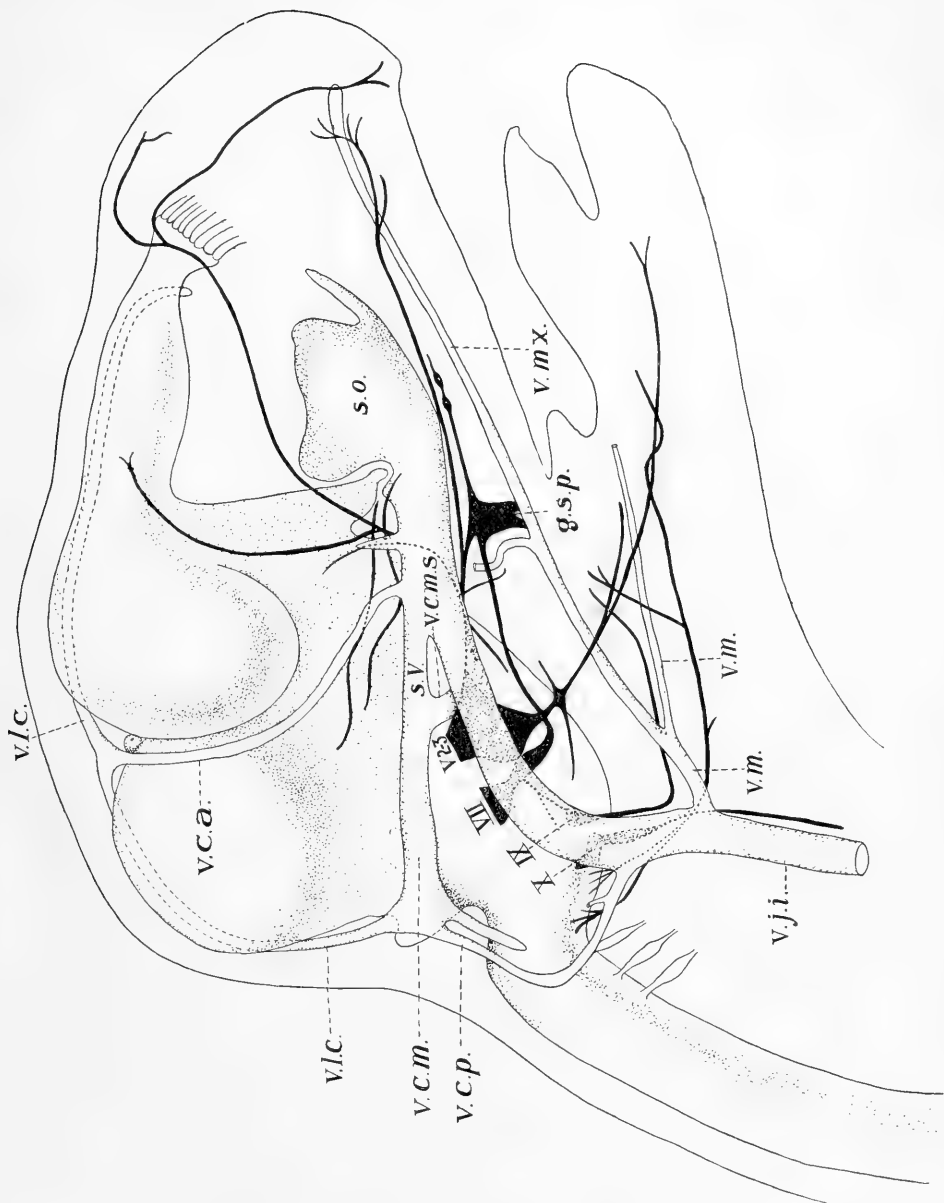


Fig. 1. Cephalic veins of a late embryo of *Tropidonotus natrix*, head 7.5 mm. long. $\times 24$. After Grosser and Brezina, 95.

g. s. p., ganglion sphenopalatinum; *s. o.*, sinus orbitalis; *s. V.*, "secundäre Verbindung" (see text); *v. c. a.*, vena cerebralis anterior; *v. c. m.*, vena cerebralis media; *v. c. m. s.*, vena cerebralis media secunda; *v. l. c.*, vena longitudinalis cerebri; *v. c. p.*, vena cerebralis posterior; *v. j. i.*, vena jugularis interna; *v. m.*, vena mandibularis; *v. m. x.*, vena maxillaris; *V. 2-3*, ganglion of second and third branches of trigeminus; *VII, IX, X*, cranial nerves.

I. THE CEPHALIC VEINS AND SINUSES OF *LACERTA AGILIS*.

Vogt and Jung, 89-94, have recognized three chief veins in the head of *Lacerta ocellata*, a "supraorbital," an "infraorbital," and a "jugular," but the description of these veins is very meager. More or less incomplete observations on isolated vessels have also been made by different authors, whose descriptions, based on different species, are referred to later. The cephalic veins of *Lacerta agilis* have been studied by Grosser and Brezina, 95, who have described in an excellent paper, the development of the larger veins, including both the primitive arrangement outlined above and the changes which occur in later embryonic stages.

The following description of adult conditions is based chiefly on *Lacerta agilis*, but it will apply also with little modification to the American lizard, *Cnemidophorus sexlineatus*, which is occasionally referred to by way of comparison. Other forms are also utilized in connection with the study of special parts.

The head of *Lacerta* includes two almost distinct venous territories: (1) A dorsal one which includes the face and cranium and is drained by the vena jugularis interna; (2) a ventral territory which includes the tongue, larynx, pharynx, trachea, and the floor of the mouth. It is drained by the vena trachealis.

A. THE TERRITORY OF THE VENA JUGULARIS INTERNA.

The cervical part of the vena jugularis interna of lizards is briefly described by Corti, 41, Parker, 84, and Vogt and Jung, 89-94. The cephalic portion of the vein is probably included in the vena infra-orbitalis of Vogt and Jung, 89-94, p. 714, although its relations are inaccurately shown in their Fig. 290, p. 712. The anterior part of the vein has been well worked out by Grosser and Brezina, 95.

The authors last mentioned have shown that in the earlier embryonic stages of the lizard the vena jugularis interna (vena cardinalis) lies on the ventral side of all cranial nerve trunks. In later stages the anterior part of the vein retains this primitive relation, while the post-trigeminal portion shifts its position to the dorsal (lateral) side of the posterior nerves, the change being effected by ring formation around the roots of the nerves, and subsequent obliteration of the ventral part of the rings. Grosser and Brezina retain for the subneural vessel the name vena cardinalis, while the posterior part of the vein is called vena capitis lateralis. In the following account I shall use the name vena jugularis

interna to include both the vena cardinalis and its physiological successor, the vena capitis lateralis.

In adult *Lacerta agilis* the vena jugularis interna (*v. j. i.*, Text Fig. 2) arises from the posterior median portion of the sinus orbitalis, a great blood-space formed by the enlargement of certain veins of the orbit. The transition from the sinus orbitalis to the vein is a gradual one, for the vein itself is much enlarged for some distance behind the orbit. For descriptive purposes the sinus may be said to terminate at the optic chiasma, or more accurately, at the rostral margin of a cartilaginous plate, the subiculum infundibuli of Gaupp, **oo**, which crosses the median line just behind the chiasma (*S. i.*, Fig. 5, Plate I). At this point the sinus orbitalis shows a horizontal portion between the bursalis muscle and the oral mucous membrane, and a vertical portion which lies between the eye muscles and the muscles which fill the temporal fossa. The vertical part of the sinus reaches dorsad as far as the ramus frontalis ophthalmicus V. At the rostral margin of the subiculum infundibuli the ventral part of the sinus gives rise to the vena jugularis interna, while the vertical part is connected with a short vein, called by Grosser and Brezina "secondäre Verbindung" of the vena cerebralis media (*s. V.*, Fig. 5, Plate I; Text Fig. 2).

From its origin the vena jugularis interna maintains a straight course toward the basisphenoid bone, which it meets lateral to the hypophysis. Throughout this stretch the vein is much flattened between the eye muscles and the adjacent parts, the main channel lying next to the middle line, while the lateral portion has an irregular and somewhat indefinite border (compare Figs. 4 and 5, Plate I). At the level of the posterior margin of the subiculum infundibuli the two venæ jugulares are connected across the middle line by a short anastomosis, which passes between the floor of the cranium and the cartilaginous basis cranii, a median strip of cartilage formed by the fusion of the trabeculæ cranii. Behind this anastomosis the vena jugularis expands on the lateral aspect of the eye muscles and reaches the ramus ophthalmicus V. Just in front of the basisphenoid bone the lateral part of the vein receives from the foramen trigemini the vena cerebralis media secunda of Grosser and Brezina (*v. c. m. s.*, Text Fig. 2).

At the rostral end of the basisphenoid bone the character of the vena jugularis interna is changed and the sinus-like vein, which has been hitherto practically a continuation of the sinus orbitalis, acquires a more definite wall and assumes the proportions of an ordinary vein. The reduced vein, which is continuous with the median part of the enlarged

vessel, diverges from the middle line and makes its way, through a notch in the basisphenoid bone, to the lateral aspect of the Eustachian furrow, along which it ascends toward the roof of the tympanic cavity. Directly anterior to the tympanum the vein receives a large anastomotic vessel from the lower jaw, the vena tympanica anterior, after which it passes between the quadrate bone and the ear capsule and enters the roof of the tympanum. Here the vena jugularis interna runs on the dorsal side of the ramus posterior facialis¹ (*r. p. f.*, Text Figs. 2 and 7). Continuing caudad the vein passes between the posterior end of the parotic process and the cranial end of the second epibranchial cartilage of Parker, 84, the latter of which lies between the vein and the trunks of the posterior cranial nerves (*IX, X, XI*, Fig. 3, Plate I). Here the vena jugularis interna receives the vena mandibularis, which approaches the trunk vein from a lateral direction (*v. m.*, Text Figs. 2 and 3). Close behind the junction of these veins the vena cerebialis posterior passes over the epibranchial cartilage from a medial direction and enters the jugular vein from above. In this region the vena jugularis interna is surrounded by a small muscle, *m. constrictor venæ jugularis interna*, which is described in the second part of this paper.

Behind the junction of the vena mandibularis with the jugular vein the cranial nerves which lie on the median side of the epibranchial cartilage begin to separate, the vagus following the jugular vein, the glosso-pharyngeus and hypoglossus running below the vein, as described by Grosser and Brezina. The trunk of the accessorius, on the other hand, passes above the jugular vein to reach its peripheral territory, the *m. cucullaris* and *m. episterno-cleido-mastoideus*. This nerve, therefore, forms an exception to the rule that the post-trigeminal nerve trunks of the adult *Lacerta agilis* lie ventral or medial to the vena jugularis interna.

In the neck region the vena jugularis interna runs on the median side of the *m. episterno-cleido-mastoideus*, where it lies close to the vagus and the carotid artery. Approaching the heart the vein receives the vena jugularis externa then bends mesad to discharge into the vena cava anterior.

¹ In *Platydactylus* the vena jugularis interna shows a tendency to retain its primitive relation to this nerve and to the chorda tympani, the latter of which becomes an independent nerve near the level of the ganglion facialis. In two specimens examined the chorda tympani passes above the vein. In one of these the ramus posterior facialis takes a similar course.

In a specimen of *Anguis fragilis* the vein lies below the auditory nerve but above the ramus posterior facialis.

The chief tributaries of the vena jugularis interna are: (a) The sinus orbitalis, (b) vena pterygoidea, (c) vena cerebrealis media, (d) vena tympanica anterior, (e) vena mandibularis, (f) vena cerebrealis posterior, (g) vena jugularis externa.

a. THE SINUS ORBITALIS.

(*s. o.*, Text Fig. 2; Fig. 1, Plate II.)

A description of the sinus orbitalis must be to some extent a repetition of the excellent account of Weber, 77, who, in fact, has been followed by all later authors. Weber, however, studied the sinus apart from the system to which it belongs, for he made no attempt to describe its tributaries or its drainage. The development of the sinus orbitalis is described by Grosser and Brezina, 95, who, also, observed its relation to the vena jugularis interna.

In *Lacerta* the sinus orbitalis occupies the space between the bulbus and the orbital walls, and surrounds the various structures which extend into this space. The sinus reaches its greatest development in the region of the rectus inferior and obliquus inferior, whence it spreads through the median, ventral, and posterior parts of the orbit. The posterior wall of the sinus is a fascia which separates the orbit from the great temporal fossa; its median boundary is the septum interorbitale, which the sinus follows forward to the fissura orbito-nasalis Gaupp, 00. The floor of the sinus is formed immediately by the smooth orbital muscle of Leydig, 72, below which lies the m. depressor palpebræ inferioris, Weber, 77. The sinus follows these muscles into the lower eyelid which it penetrates as far as the lower margin of the tarsal plate.

In the rostral part of the orbit the sinus extends into the membrana nictitans, where it communicates with a small *sinus membranæ nictitantis* (*s. m. n.*, Text Fig. 2). This sinus lies partly between the tubules of the Harderian gland, partly in the non-glandular portion of the lid near its free border.

In a dorsal direction the sinus orbitalis forms an incomplete covering for the bulbus, only the posterior part of the sinus rising to the roof of the orbit. The median part of the sinus reaches the floor of the cranium, and expands laterally as far as the tænia marginalis, a band of cartilage which lies in the lateral wall of the cranium (Gaupp, 00). From this part of the sinus enlarged capillaries continue laterad above the bulbus and communicate with the dorsal portion of the sinus membrana nictitantis.

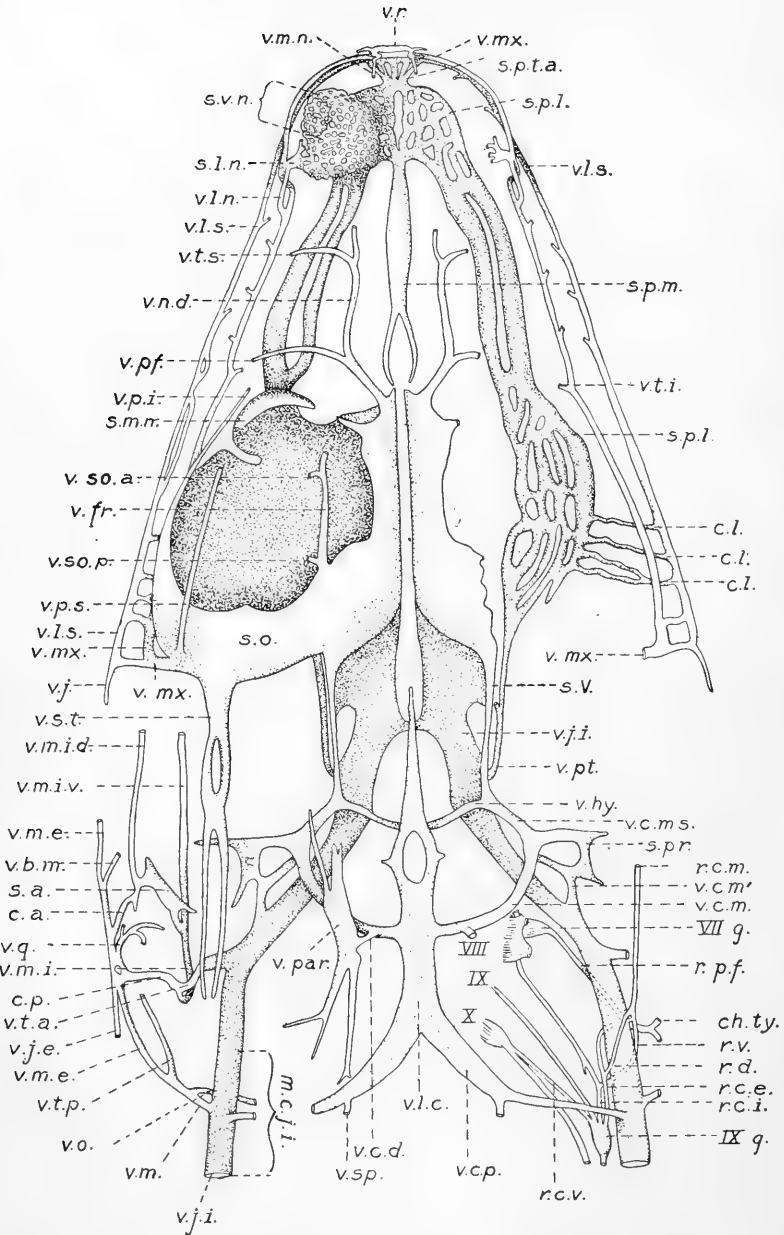


FIG. 2. The cephalic veins and sinuses of adult *Lacerta agilis*, total length 18 cm. Dorsal view. $\times 9$.

c. a., *c. l.*, *c. p.*, anastomotic veins described in the text; *ch. ty.*, chorda tympani; *m. c. j. i.*, the dotted line indicates the position of the *m. con-*

The median part of the sinus orbitalis extends backward below the floor of the cranium until it reaches the posterior margin of the cartilaginous plate, *solum suprasedale* Gaupp, behind which the sinus is invaded by the optic nerves. At the level of the chiasma the sinus is continuous with the vena jugularis interna and the secondary connection of the vena cerebialis media, as previously described.

Into the sinus orbitalis discharge practically all of the veins of the anterior region of the head, and one large postorbital vein. The chief tributaries are the following: (1) Vena maxillaris, (2) vena nasalis dorsalis, (3) vena frontalis, (4) vena subseptalis, (5) venæ palpebrales, (6) vena supratemporalis, (7) secondary connection of the vena cerebialis media.

1. VENA MAXILLARIS (*v. mx.*, Text Figs. 2 and 3; Figs. 2 and 3, Plate II).—The vena maxillaris of *Lacerta agilis* begins in the vena rostralis (*v. r.*, Text Fig. 2), a subcutaneous vein which forms an irregular ring about the rostrum and receives small tributaries from the skin and deeper parts. Through the foramen apicale the vena rostralis receives on each side a vena medialis nasi (*v. m. n.*, Text Fig. 2), which drains the tissues immediately lateral to the septum nasale. The chief tributary of the medial vein is the vena suprasedalis lateralis, which lies on the dorsal aspect of the septum nasale, where it is formed by the bifurcation of a vena suprasedalis media (*v. s. m.*, Figs. 2 and 3, Plate II).

strictor venæ jugularis internæ; *r. c. e.*, *r. c. i.*, *r. c. m.*, *r. c. v.*, *r. d.*, *r. v.*, nerves described in the text; *s. a.*, sinus articularis; *s. l. n.*, sinus lateralis nasi; *s. m. n.*, sinus membranæ nictitantis; *s. o.*, sinus orbitalis; *s. p. l.*, sinus palatinus lateralis; *s. p. m.*, sinus palatinus medius; *s. p. t. a.*, sinus palatinus transversus anterior; *s. pr.*, sinus prooticus; *s. V.*, secondary connection of the vena cerebialis media; *s. v. n.*, sinus vestibuli nasi; *v. b. m.*, vena bucco-mandibularis; *v. c. d.*, vena capitis dorsalis; *v. c. m.*, vena cerebialis media; *v. c. m'*, primary connection of the vena cerebialis media with the vena jugularis interna; *v. c. m. s.*, vena cerebialis media secunda; *v. c. p.*, vena cerebialis posterior; *v. fr.*, vena frontalis; *v. hy.*, vena hypophyseos; *v. j.*, vena jugalis; *v. j. e.*, vena jugularis externa; *v. j. i.*, vena jugularis interna; *v. l. c.*, vena longitudinalis cerebri; *v. l. n.*, vena lateralis nasi; *v. l. s.*, vena labialis superior; *v. m.*, vena mandibularis; *v. m. e.*, vena mandibularis externa; *v. m. i.*, vena mandibularis interna; *v. m. i. d.*, dorsal part of the vena mandibularis interna; *v. m. i. v.*, ventral part of the vena mandibularis interna; *v. m. n.*, vena medialis nasi; *v. mx.*, vena maxillaris; *v. n. d.*, vena nasalis dorsalis; *v. o.*, vena œsophagea; *v. par.*, vena parietalis; *v. p. i.*, vena palpebralis inferior; *v. p. s.*, vena palpebralis superior; *v. pf.*, vena præfrontalis; *v. pt.*, vena pterygoidea; *v. q.*, vena quadrata; *v. r.*, vena rostralis; *v. so. a.*, vena supraorbitalis anterior; *v. so. p.*, vena supraorbitalis posterior; *v. s. t.*, vena supratemporalis; *v. sp.*, vena spinalis; *v. t. a.*, vena tympanica anterior; *v. t. i.*, vena turbinalis inferior; *v. t. p.*, vena tympanica posterior; *v. t. s.*, vena turbinalis superior; VIII, IX, X, roots of cranial nerves; VIIg., ganglion facialis; IXg., ganglion glossopharyngei.

The ventral part of the vena rostralis is connected by several short anastomoses with the sinus palatinus, the connecting veins passing through the dentary portion of the intermaxillary bone. The lateral portion of the vena rostralis gives rise, on each side, to a vena maxillaris and a vena labialis superior (Text Fig. 2).

The *vena maxillaris*, which is the more dorsal of these veins, passes under the external nasal opening and runs caudad for a short distance on the lateral aspect of the maxillary bone (*v. mx.*, Figs. 2 and 3, Plate II). At the level of the posterior end of the nasal vestibule the vein passes under the small *sinus lateralis nasi* (*s. l. n.*, Text Fig. 2), with which the vein is connected by a short anastomosis. Continuing caudad the vein enters the maxillary bone, where it is joined by the *vena lateralis nasi* (*v. l. n.*), which also drains the sinus lateralis nasi. Near the caudal end of the maxillary bone the vena maxillaris is joined by an anastomotic vein from the vena labialis superior, after which it emerges on the dorsal surface of the alveolar portion of the bone. Here it receives the *vena turbinalis inferior* (*v. t. i.*, Text Fig. 2), which drains the lateral part of the turbinal prominence and the region adjacent to the posterior end of the ductus naso-lachrymalis. A little farther caudad the vena maxillaris enters the jugal bone, where it receives one or two anastomotic veins from the vena labialis superior. At the angle of the mouth the vena maxillaris escapes from the bone and forms a junction with the vena labialis superior, after which it bends dorsad, between the jugal bone and the ramus maxillaris V, and discharges into the posterior part of the sinus orbitalis.

In *Lacerta agilis* the vena maxillaris terminates in the sinus orbitalis and has no connection with the postorbital veins.

The chief tributaries of the vena maxillaris are the sinus vestibuli nasi and the vena labialis superior, which are reserved for a separate description.

(1) *Sinus Vestibuli Nasi*.—The nasal vestibule, or anterior part of the nasal cavity of the lizard, is a passage of considerable length, which is lined with stratified squamous epithelium and surrounded, outside of the epithelium, by a thick spongy layer resembling erectile tissue. The sinus vestibuli nasi (*s. v. n.*, Text Fig. 2; Figs. 2 and 3, Plate II) includes the communicating blood-spaces of this spongy stratum. The blood-spaces are lined with a simple endothelium and seem to represent enlarged capillaries or veins. They are separated by trabeculae composed of connective tissue and smooth muscle fibers (*m.*, Fig. 5, Plate II), the latter arranged in a radial direction with reference to the nasal

vestibule. The spongy tissue begins at the external nasal opening and extends through the entire length of the vestibule. The blood-spaces are fed, in part at least, by small arteries from the a. medialis nasi, which runs along the median wall of the nasal vestibule in company with the ramus medialis nasi ophthalmicus V and the vena medialis nasi.

The sinus vestibuli nasi is drained by two or three short veins which run directly laterad from the posterior part of the sinus. These veins discharge into the sinus lateralis nasi, which in turn is drained by the vena maxillaris and the vena lateralis nasi, as already described.

The literature dealing with the sinus vestibuli nasi is referred to on page 70; the function of the spongy tissue is described later.

(2) *Vena Labialis Superior* (*v. l. s.*, Text Fig. 2).—The vena labialis superior begins in the vena rostralis immediately ventral to the origin of the vena maxillaris. Behind their origin the two veins separate, the labial vein running close to the inner epithelium of the lip (*v. l. s.*, Figs. 2 and 3, Plate II), while the vena maxillaris takes the more dorsal course already described. In its passage caudalward the labial vein receives small tributaries from the skin and deeper parts; it is also connected by several anastomoses with the vena maxillaris. Near its posterior end the labial vein communicates with the sinus palatinus by three or four transverse veins (*c. l.*, Text Fig. 2) which run through the submucosa posterior to the teeth. One of these veins passes below the posterior end of the maxillary bone, one or two near the maxillo-jugal suture, and another under the anterior part of the jugal bone.

At the angle of the mouth the vena labialis superior bends dorsad and discharges into the vena maxillaris. Near its termination it receives a small *vena jugalis*, which drains a fold extending backward from the angle of the mouth. The fold contains a process of the jugal bone.

2. *VENA NASALIS DORSALIS* (*v. n. d.*, Text Fig. 2).—The vena nasalis dorsalis arises in the skin which covers the nasal bones. Anterior to the fronto-nasal suture it passes through the nasal bone and runs backward, first between the bones and the cartilaginous roof of the nasal capsule, then through the lateral portion of the fenestra olfactoria, where the vein lies in the cranial wall close to the roof cartilage. Continuing caudad the vein passes under the cartilago sphen-ethmoidea, which it follows to the fissura orbito-nasalis, through which the vein discharges into the sinus orbitalis. The vena nasalis dorsalis has the following tributaries:

(1) *Vena Turbinalis Superior* (*v. t. s.*, Text Fig. 2).—This vein drains the dorsal part of the concha and leaves the nasal cavity through the posterior part of the fenestra lateralis nasi of Gaupp, oo. Outside of

the nasal cavity the vein runs mesad above the cartilaginous roof of the nasal capsule and joins the vena nasalis dorsalis at the posterior end of the fenestra olfactoria.

(2) *Vena Præfrontalis* (*v. pf.*, Text Fig. 2).—This vein drains the præfrontal bone and the adjacent part of the orbital wall. It has its origin in the wall of the orbit, whence it runs mesad through the præfrontal bone and joins the vena nasalis dorsalis below the junction of the sphen-ethmoid cartilage with the roof of the nasal capsule (tectum nasale).

The system of the vena nasalis dorsalis is paralleled by a system of arteries and nerves, whose chief trunks, the ethmoid artery and nerve, pass through the fissura orbito-nasalis above the vena nasalis dorsalis.

3. *VENA FRONTALIS* (*v. fr.*, Text Fig. 2).—The vena frontalis drains the frontal and supraorbital bones and the adjoining skin. The vein is formed by two cutaneous roots, vena frontalis anterior and vena frontalis posterior, which run through the frontal bone and unite near the fronto-parietal suture. The trunk vein runs directly caudad under the lateral portion of the parietal bone and on the lateral aspect of the tения marginalis of the chondrocranium. Caudal to the junction of the tения marginalis with the suprasedal cartilage (solum suprasedale) the vein discharges into the dorsal part of the sinus orbitalis.

The vena frontalis receives two tributaries from the supraorbital region:

(1) *Vena Supraorbitalis Anterior* (*v. so. a.*, Text Fig. 2).—This vein collects the blood from the rostral portion of the supraorbital region. It joins the vena frontalis near the fronto-parietal suture.

(2) *Vena Supraorbitalis Posterior* (*v. so. p.*, Text Fig. 2).—This vein receives blood from the posterior part of the supraorbital area; it joins the vena frontalis just anterior to the termination of the latter in the sinus orbitalis.

The entire system of the vena frontalis is paralleled by a system of arteries and nerves which arise, respectively, from the arteria supraorbitalis and the ramus frontalis ophthalmicus V.

4. *VENA SUBSEPTALIS*.—The vena subseptalis is a short vein which begins at the posterior end of the cartilago paraseptalis of Gaupp, oo. It runs forward and dorsad a short distance and discharges into the sinus orbitalis through the ventral portion of the fissura orbito-nasalis.

The vena subseptalis is formed on each side by the union of two veins, the *vena subseptalis anterior* and *vena subseptalis posterior*. The two venæ subseptales anteriores have a common origin in a *vena subseptalis*

media, which begins between the openings of Jacobson's organ and runs caudad in the median line, between the foot of the septum nasale and the median vomerine suture. Between the choanae the vena subseptalis media divides into the two venae subseptales anteriores, which run caudad, one on each side of the median line, between the paraseptal cartilage and the foot of the septum nasale. Near the posterior end of the paraseptal cartilage the vena subseptalis anterior receives from the choana a small tributary which drains the median part of the floor of the nasal cavity.

The vena subseptalis posterior begins below the septum interorbitale near the posterior end of the palatine bone; it runs forward near the median line and joins the vena subseptalis anterior at the posterior end of the paraseptal cartilage, as described above.

5. VENÆ PALPEBRALES. (1) *Vena Palpebralis Inferior* (*v. p. i.*, Text Fig. 2).—This vein has its origin under the orbital end of the ductus naso-lachrymalis and runs caudad along the ventral margin of the tarsus of the lower eyelid. As it approaches the posterior canthus the vein enlarges gradually and discharges into the palpebral portion of the sinus orbitalis.

(2) *Vena Palpebralis Superior* (*v. p. s.*, Text Fig. 2).—This vein begins near the anterior canthus and runs caudad through the deeper part of the cutis plate near the proximal border of the upper eyelid. Behind the level of the posterior canthus the vein runs for a short distance between the cutis and Leydig's smooth muscle, through the latter of which it finally breaks and discharges into the posterior part of the sinus orbitalis.

6. VENA SUPRATEMPORALIS (*v. s. t.*, Text Fig. 2).—The vena supratemporalis is a subcutaneous vein which runs over the top of the head in company with the arteria temporo-muscularis and the ramus recurrens n. maxillaris ad n. facialem of Fischer, 52. The vein begins above the parotic process, in the region between the parotic portion of the parietal bone and the squamosum of Parker, 84. From this region two parallel, freely anastomosing veins run forward between the m. temporalis and the skin. Anteriorly these veins unite to form a sinus-like trunk, which continues forward and discharges into the dorsal part of the sinus orbitalis.

The vena supratemporalis probably corresponds to the vena supra-orbitalis of Vogt and Jung, 89-94 (Fig. 290, p. 712), who, however, represent the vein as discharging into the vena jugularis interna. The anterior part of the vena supratemporalis has been observed by Weber, 77, who apparently considered it an outlet of the sinus orbitalis.

7. SECONDARY CONNECTION OF THE VENA CEREBRALIS MEDIA.—I

have little to add to Grosser and Brezina's description of this vein, whose history, as given by these authors, 95, is closely correlated with that of the vena cerebralis anterior. In earlier embryonic stages the latter is a continuous vein which extends from the vena longitudinalis cerebri to the anterior part of the vena jugularis interna (compare Text Fig. 1). In later stages the vena cerebralis anterior breaks in the middle, the dorsal portion discharging into the median vein, while the ventral part retains its relation to the vena jugularis interna. At this time the secondary connection (secundäre Verbindung) is formed, between the ventral part of the vena cerebralis anterior and the vena cerebralis media secunda. In later stages the ventral part of the vena cerebralis anterior is drowned by the posterior extension of the sinus orbitalis. Hence, in the adult lizard the secondary connection acquires a direct outlet into the sinus orbitalis (*s. V.*, Text Fig. 2; Fig. 5, Plate I).

The dorsal part of the vena cerebralis anterior has not been identified in the adult lizard. It evidently undergoes further reduction before the adult condition is reached.

b. VENA PTERYGOIDEA AND THE SINUS PALATINUS.

1. VENA PTERYGOIDEA (*v. pt.*, Text Fig. 2).—This vein begins at the posterior border of the foramen suborbitalis, where it receives the drainage of the posterior part of the sinus palatinus lateralis. The vein runs caudad on the dorsal side of the pterygoid bone (*v. pt.*, Fig. 4, Plate I) until it reaches the foot of the columella (os epipterygoideum), on whose median side the vein bends dorsad and enters the vena jugularis interna.

The vena pterygoidea receives small tributaries from the pterygoid bone and from the oral mucous membrane; its most important tributary, however, is the sinus palatinus.

2. SINUS PALATINUS (Text Fig. 2; Figs. 2 and 3, Plate II).—This sinus lies in the submucosa of the roof of the mouth. It begins near the rostrum and extends caudad, on each side of the head, as far as the pterygo-transverse bar which forms the posterior boundary of the foramen suborbitalis. The sinus includes a sinus palatinus medius, the paired sinus palatinus lateralis, and two sinus palatini transversi.

The most anterior part of the system is the *sinus palatinus transversus anterior* (*s. p. t. a.*, Text Fig. 2), which lies behind the dentary portion of the intermaxillary bones, through which it communicates with the vena rostralis, as elsewhere described. The sinus palatinus transversus anterior gives rise on each side to a *sinus palatinus lateralis* (*s. p. l.*, Text Fig. 2; Figs. 2 and 3, Plate II), which forms, directly behind its

origin, a system of closely anastomosing vessels, which extend from the dental furrow almost to the middle line, and caudad to the openings of Jacobson's organ. In front of these openings the two sinus palatini laterales are again connected across the middle line by a short transverse vessel, *sinus palatinus transversus posterior*. Into the last discharges the *sinus palatinus medius* (*s. p. m.*, Text Fig. 2), which begins immediately behind the choanae and runs rostrad in the middle line.

Behind the sinus palatinus transversus posterior the two sinus palatini laterales again separate and diverge from the middle line, each sinus including two or three large vessels. In a posterior direction the number of these vessels increases to occupy the greater space made by the divergence of the jaws, the stronger vessel lying close to the dental furrow. Behind the teeth this larger vessel communicates with the vena labialis superior by three or more small vessels (*c. l.*, Text Fig. 2), which run through the submucosa of the upper jaw. Behind the level of these anastomoses the sinus palatinus lateralis is gradually reduced, both as to the number and size of its vessels, until only the vena pterygoidea remains.

The larger vessels of the sinus palatini can be readily seen in the living animal through the mucous membrane. References to these sinuses are to be found in the papers of Leydig, 72, p. 99, and Born, 79, p. 98, but no complete description is given.

c. VENA CEREBRALIS MEDIA.

(*v. c. m.*, Text Fig. 2.)

In early embryonic stages of *Lacerta agilis*, as described by Grosser and Brezina, 95, the vena cerebralis media discharges into the vena jugularis interna through the posterior part of the foramen trigemini. Later the extracranial part of the vein is lost and a more anterior outlet is formed, the vena cerebralis media secunda, which runs from the anterior part of the foramen trigemini and joins the vena jugularis interna below the cranium. The same authors also state, that with the approach of adult life the vena cerebralis media is broken into two parts by the degeneration of its middle section. I have been unable to find any evidence of degeneration in my specimens of *Lacerta agilis*. A continuous vena cerebralis media also occurs in a specimen of *Agama colorum*, in which the entire vein is very conspicuous because of a natural injection of blood.

In adult *Lacerta* the vena cerebralis media arises from the vena longi-

tudinalis cerebri under the crista sagittalis of the superior occipital bone. From its origin the vein runs laterad until it reaches the prominencia vestibularis interna, on whose dorsal aspect it runs forward to the foramen trigemini (incisura prootica of Gaupp, oo). Here the vein forms a *sinus prooticus*, which surrounds the ganglion of the maxillary and mandibular branches of the trigeminus, excepting in a dorsal direction, where the ganglion lies in contact with the prootic bone. Laterally the sinus is bounded by the m. temporalis, between which and the m. pterygoideus internus the sinus extends as far as the pterygoid bone.

From the rostral part of the sinus prooticus an extracranial vein, the *vena cerebialis media secunda* (*v. c. m. s.*, Text Fig. 2), runs ventrad and enters the vena jugularis interna just in front of the basisphenoid bone. In one of the adult specimens examined during the preparation of this paper the posterior outlet of the vena cerebialis media also persists as a small vein (*v. c. m.*, Text Fig. 2), although it is probably obliterated as a rule.

The vena cerebialis media secunda is connected with the sinus orbitalis by a small vein which runs along the ventral aspect of the ramus ophthalmicus V. It is the secondary connection (secundäre Verbindung) which is described on page 16. In the adult lizard this vein is of little importance and it may be doubted if it carries blood to the sinus orbitalis under ordinary conditions.

The sinus prooticus also receives several small veins from the m. temporalis, the most important of which begins near the articulation of the jaw and runs dorsad on the lateral aspect of the pterygoid bone. In an embryo *Lacerta* with head 5.2 mm. long this vein is connected with the vena mandibularis interna. The same relation has also been observed in advanced embryos of *Cnemidophorus*.

The most important tributaries of the vena cerebialis media are the vena capitis dorsalis and vena hypophyseos.

1. *VENA CAPITIS DORSALIS* (*v. c. d.*, Text Fig. 2).—The vena capitis dorsalis drains the muscles of the occipital fossa, including the cephalic portions of the mm. capiti-cervicales and the posterior dorsal portion of the m. temporalis. The vein is formed by the union of two roots, a lateral one which arises above the parotic process, and a median one which has its origin above the lateral margin of the foramen magnum. These two veins run forward under the m. capiti-cervicalis superior and unite below the posterior margin of the parietal bone, directly lateral to the crista sagittalis of the supraoccipital. The sinus-like trunk vein continues forward a short distance, then bends toward the median line and

enters the cranium through the caudal end of the great parietal fissure, which lies between the parietal bone and the dorsal margin of the prootic. Within the cranium the vein joins the vena cerebialis media close to the origin of the latter from the vena longitudinalis cerebri.

Before it enters the cranial cavity the vena capitis dorsalis receives, from an anterior direction, a sinus-like vein, vena parietalis (*v. par.*, Text Fig. 2), which lies external to the membrane which closes the parietal fissure. The vein is formed by the union of two tributaries, vena parietalis dorsalis and vena parietalis ventralis. The former is an enlarged vein which lies close to the cranial wall near the roof of the head. It begins near the level of the pineal body. The vena parietalis ventralis begins somewhat farther forward but is a smaller vein than the preceding. It runs caudad on the ventro-lateral aspect of the cartilaginous rod, tænia marginalis of Gaupp, oo, which lies in the cranial wall dorsal to the prootic bone. Anterior to the caudal end of that cartilage the two venæ parietales unite, the trunk vein, vena parietalis, running caudad until it reaches the posterior margin of the parietal bone, where it unites with the vena capitis dorsalis.

The vena capitis dorsalis is apparently referred to by Grosser and Brezina, 95, in their description of an adult *Varanus arenarius*, in which, however, the vein is said to discharge into the vena cerebialis posterior. In *Lacerta agilis* the vena capitis dorsalis has no such connection.

2. VENA HYPOPHYSEOS (*v. hy.*, Text Fig. 2).—The vena hypophyseos is a short paired vein which is formed behind the hypophysis by the forking of the vena infundibuli. From its origin the vein runs laterad and rostrad around the hypophysis until it reaches the anterior end of the basisphenoid bone, where the vein enters the vena cerebialis media secunda just above the junction of the latter with the vena jugularis interna. The chief tributary of the venæ hypophyseos is the *vena infundibuli*, a median vein which lies on the posterior aspect of the infundibulum. It is formed by the union of the right and left venæ thalamencephali, which receive blood from the floor and lateral wall of the third ventricle.

d. VENA TYMPANICA ANTERIOR.

The vena tympanica anterior (*v. t. a.*, Text Fig. 2) is an anastomotic vein which connects the vena jugularis interna and the vena mandibularis interna. It unites with the latter vein directly in front of the tympanum, where the vena mandibularis interna emerges from the mandible. From its origin the vena tympanica anterior runs dorsad

close to the wall of the tympanic cavity and on the median side of the chorda tympani. Just before it reaches the roof of the tympanum the vein meets the vena jugularis interna which it enters from a lateral direction.

e. VENA MANDIBULARIS.

(*v. m.*, Text. Figs. 2 and 3).

The vena mandibularis of *Lacerta agilis* is referred to by Grosser and Brezina, 95, in their description of embryo VI (head 3.1 mm.) under the name vena maxillaris. The vein is considered a temporary structure, however, for in their description of a later stage (embryo XIV, head 4.1 mm. long) it is said to have found a substitute in the vena trachealis.

In my sections of adult *Lacerta agilis* and other lizards the vena mandibularis is well developed, though it is a vein of only moderate size. Under typical conditions, such as we find in *Cnemidophorus*, the vena mandibularis is formed by the union of two veins, the vena mandibularis interna and vena mandibularis externa, which unite at a point dorsal to the mandible and posterior to the tympanum. In *Lacerta* the vena mandibularis interna fails to join the vena mandibularis externa, but I shall, nevertheless, consider the vena mandibularis as beginning at the same point where it begins in *Cnemidophorus*. From this point, which is designated above, the vena mandibularis of *Lacerta* runs dorsad toward the vena jugularis interna, which it enters from a lateral direction, a short distance anterior to the mouth of the vena cerebralis posterior (compare Text Figs. 2 and 3; Fig. 3, Plate I).

In addition to the vena mandibularis externa, which is described below, the vena mandibularis of *Lacerta* receives two other tributaries (Text Fig. 2): a vena *oesophagea* and a vena *tympanica posterior*. The former (*v. o.*) drains the mucous membrane of the oesophagus, together with the adjacent muscles. The vena tympanica posterior (*v. t. p.*) receives blood from the floor and posterior wall of the tympanic cavity. It runs caudad along the ventral margin of the tympanic membrane and joins the vena mandibularis a short distance above the posterior end of the mandible.

The internal and external mandibular veins must now be described.

1. VENA MANDIBULARIS INTERNA (*v. m. i.*, Text Fig. 2).—The vena mandibularis interna of *Lacerta* is, for the most part, an intrasosseous vein, which begins in the anterior part of the mandible and passes backward on the dorsal aspect of Meckel's cartilage in company with the

mandibular artery and nerve. In the anterior part of its course the vein forms occasional anastomoses with the vena mandibularis externa which lies laterad from the mandible. Near the angle of the mouth the vena mandibularis interna divides into two parts: a *pars dorsalis* (*v. m. i. d.*, Text Figs. 2 and 3), which runs caudad through the dorsal part of the mandible, and a *pars ventralis* (*v. m. i. v.*, Text Figs. 2 and 3), which follows Meckel's cartilage and the ramus mandibularis V. In front of the articulation of the jaw the pars dorsalis enters a large foramen, *f. articulare*, which penetrates the mandible in a transverse direction and furnishes a passage way for the vena communicans anterior, the ramus recurrens cutaneous mandibularis V. and a small artery. Here the pars dorsalis meets the vena communicans anterior, an anastomotic vein from the vena mandibularis externa, then expands to form the *sinus articularis* (*s. a.*, Text Figs. 2 and 3), a small sinus which lies in a concavity on the medial side of the mandible just below the articulation.

From the caudal part of the sinus articularis the pars dorsalis of the vena mandibularis interna again enters the mandible and joins the pars ventralis, which approaches the articulation accompanied by the chorda tympani. The reconstructed vein runs caudad, accompanied by the chorda tympani, until it reaches a point posterior to the articulation, where the vein and nerve issue through the same foramen upon the dorsal surface of the mandible. Here the vena mandibularis interna forms a junction with two anastomotic veins: a peripheral one and a central one. The larger central vein is the *vena tympanica anterior* (*v. t. a.*, Text Figs. 2 and 3), which runs dorsad close to the anterior wall of the tympanic cavity and on the median side of the chorda tympani. The vein enters the vena jugularis interna near the roof of the tympanic cavity. The peripheral vein, *vena communicans posterior*, is a short transverse vessel which is connected laterally with the vena mandibularis externa.

At its junction with the vena tympanica anterior and vena communicans posterior the vena mandibularis interna of *Lacerta* ends. This condition is probably not a primitive one, however, for in other lizards, as previously stated, the vein joins the vena mandibularis externa behind the tympanum. The change which has occurred in *Lacerta* will be more apparent after a brief description of the vena mandibularis interna of *Cnemidophorus* and *Agama*.

In mature embryos of *Cnemidophorus sexlineatus* I find, in front of the articulation of the jaw, a sinus articularis, which is connected anastomotically with the vena mandibularis externa and also with the sinus

prooticus. From the sinus articularis the vena mandibularis interna runs caudad below the articulation, posterior to which it again forms two anastomoses, one with the vena mandibularis externa, the other with the vena jugularis interna. From this point the vein continues caudad on the dorsal aspect of the mandible to join the vena mandibularis externa behind the tympanic cavity.

In Agama the sinus articularis lies dorsal to the trough-shaped mandible. It is connected with the vena mandibularis externa by two anastomoses, one of which passes through the foramen articulare, while the other runs above the lateral margin of the mandible. From a median direction the sinus also receives a short vein which takes its origin near the sinus prooticus; it corresponds to the peripheral part of the vein which connects the sinus prooticus and the sinus articularis in Cnemidophorus. In front of the articulation the vena mandibularis interna of Agama divides into two parts, one of which runs through the mandible below the articulation, while the other, larger division runs along the median side of the mandible. Behind the articulation the two veins unite and the trunk vein is connected anastomotically with the vena mandibularis externa, as in Cnemidophorus. In Agama a prætympanic connection between the vena mandibularis interna and the vena jugularis interna is wanting, but the vena tympanica anterior is represented by a short vein which drains the anterior wall of the tympanum. Proceeding caudalward, the vena mandibularis interna runs between the floor of the tympanum and the mandible and joins the vena mandibularis externa as in Cnemidophorus.

From the foregoing accounts it seems probable that the posterior prolongation of the vena mandibularis interna, as we find it in Agama and Cnemidophorus, is a primitive condition which has been modified in Lacerta by obliteration of the caudal part of the vein.

2. VENA MANDIBULARIS EXTERNA (*v. m. e.*, Text Figs. 2 and 3).—This vein begins near the symphysis of the jaw and runs caudad on the lateral aspect of the mandible until it reaches the level of the anterior wall of the tympanum. Here the vein enters the fold which forms the lower boundary of the external auditory depression, behind which it bends mesad and joins the vena mandibularis interna on the dorsal side of the articular bone.

The chief tributaries and anastomoses of the internal and external mandibular veins are the following:

(1) *Sinus Dentalis* (*s. d.*, Text Fig. 3).—This sinus lies in the submucosa internal to the dental furrow; it begins near the symphysis and

extends caudad to the posterior limit of the teeth. The outlet of the sinus has not been definitely located, but its position suggests a close relation to the mandibular veins. The sinus dentalis can be readily seen through the mucous membrane in the living *Lacerta*; it has also been observed in sections of *Agama colonorum*, in which it is well filled by a natural injection of blood.

(2) *Vena Bucco-mandibularis* (*v. b. m.*, Text Figs. 2 and 3).—This anastomotic vein arises from the vena buccalis lateral to the base of the tongue. It runs caudad and laterad, first between the mylohyoideus muscle and the oral mucous membrane, then under the mandible; it joins the vena mandibularis externa near the angle of the mouth.

(3) *Vena Communicans Anterior* (*c. a.*, Text Figs. 2 and 3).—This vein arises from the vena mandibularis externa behind the junction of the latter with the vena bucco-mandibularis. From its origin it runs through the foramen articulare and joins the sinus articularis on the median side of the mandible.

In embryos of *Lacerta* and *Cnemidophorus* the sinus articularis is also connected with the sinus prooticus, and thus continuous communication is established between the vena mandibularis externa and the vena cerebri media.

(4) *Vena Quadrata* (*v. q.*, Text Fig. 2).—This is a small vein which emerges from the anterior surface of the quadrate bone directly above the articulation of the jaw. Passing through a notch in the quadrate bone the vein reaches the lateral aspect of the articulation and joins the vena mandibularis externa. The vena quadrata has two small tributaries: one from the median side of the mandible, the other from the posterior part of the m. temporalis and the adjoining skin.

(5) *Vena Communicans Posterior* (*c. p.*, Text Figs. 2 and 3).—Lateral to the posterior part of the articulation the vena mandibularis externa forms a small ring which gives rise to the vena communicans posterior. This vein runs mesad and joins the vena mandibularis interna behind the articulation, as already described. Through the vena communicans posterior and vena tympanica anterior the vena mandibularis externa is placed in communication with the vena jugularis interna.

f. VENA CEREBRALIS POSTERIOR.

(*v. c. p.*, Text Fig. 2; Figs. 2 and 3, Plate I.)

The two venæ cerebrales posteriores are sinus-like veins which are formed by the bifurcation of the vena longitudinalis cerebri at the dorsal margin of the foramen magnum. Both veins leave the skull through the

foramen magnum and diverge from the median line, each vein bending directly laterad on its own side to reach the vena jugularis interna, which it enters from a dorsal direction.

As it issues from the skull the vena cerebialis posterior gives rise to a *vena spinalis* (*v. sp.*, Text Fig. 2), an intradural vein of small size, which runs along the lateral aspect of the spinal cord and receives intervertebral tributaries from the muscles which adjoin its path. According to the observations of Corti, 41, on *Psammosaurus*, the vena spinalis discharges into the vena cava posterior.

The only important feeder of the vena cerebialis posterior is the vena longitudinalis cerebri.

Vena Longitudinalis Cerebri (*v. l. c.*, Text Fig. 2).—This is an intradural vein which extends from the olfactory lobes to the foramen magnum. In the adult lizard it is a small vein until it reaches the epiphysis, where it receives the epiphysial veins, which enter a ring formed by the vena longitudinalis cerebri around the distal end of the epiphysial stalk. The enlarged posterior part of the vena longitudinalis cerebri gives rise to the vena cerebialis media and to the vena cerebialis posterior, as already described.

The *venæ epiphyseos* have their roots in the cerebrum, where they drain the plexus chorioideus lateralis of the lateral ventricles. From each ventricle a single vein passes through the foramen Monroi and unites with its fellow in the roof of the third ventricle. The trunk vein ascends the stalk of the epiphysis, giving rise at the same time to a number of anastomosing branches, which form the plexus chorioideus anterior. From this plexus several small veins enter the ring of the vena longitudinalis cerebri.

g. VENA JUGULARIS EXTERNA.

(*v. j. e.*, Text Figs. 2 and 3.)

The vena jugularis externa is a vein of medium size which extends from the vena mandibularis externa to the posterior end of the vena jugularis interna. The vein begins laterad of the articulation of the jaw, in a venous ring which gives rise to the vena communicans posterior. From its origin the vena jugularis externa runs through the cutaneous fold which forms the ventral margin of the external auditory depression; it then takes a direction toward the shoulder joint, in front of which it bends mesad, penetrates the superficial muscles (cucullaris and episterno-cleido-mastoideus) and finally enters the vena jugularis interna near the

termination of the latter in the vena cava anterior. Through the larger part of its course the vena jugularis externa is a subcutaneous vein, receiving numerous small tributaries from the skin.

On account of its relation to the vena mandibularis externa, the vena jugularis externa is placed in indirect communication with the vena

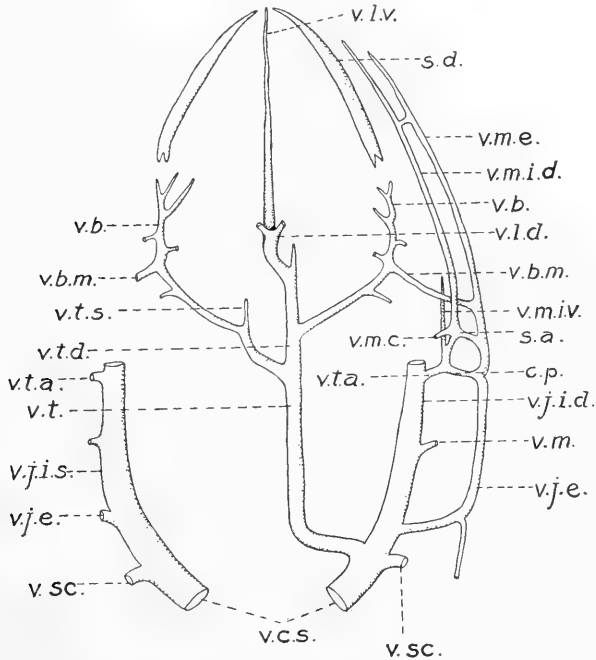


FIG. 3. Vena trachealis, vena jugularis interna and related veins of a late embryo of *Lacerta agilis* (head 5.7 mm.). $\times 10$.

c. p., posterior anastomosis between the vena mandibularis interna, and vena mandibularis externa; *s. a.*, sinus articularis; *s. d.*, sinus dentalis; *v. b.*, vena buccalis; *v. b. m.*, vena bucco-mandibularis; *v. c. s.*, vena cava superior; *v. j. e.*, vena jugularis externa; *v. j. i. d.*, vena jugularis interna dextra; *v. j. i. s.*, vena jugularis interna sinistra; *v. l. d.*, vena lingualis dorsalis; *v. l. v.*, vena lingualis ventralis; *v. m.*, vena mandibularis; *v. m. c.*, vena mandibulo-cerebralis; *v. m. e.*, vena mandibularis externa; *v. m. i. d.*, vena mandibularis interna dorsalis; *v. m. i. v.*, vena mandibularis interna ventralis; *v. s. c.*, vena subclavia; *v. t.*, vena trachealis; *v. t. a.*, vena tympanica anterior; *v. t. d.*, vena trachealis dextra; *v. t. s.*, vena trachealis sinistra.

In this stage the dorsal and ventral parts of the vena mandibularis interna are not connected anteriorly.

mandibularis interna and other deep veins, which may, therefore, find an outlet through the external jugular vein. Posteriorly the vena jugularis externa receives a cutaneous vein, which arises dorsal to the shoulder

joint; it unites with the trunk vein just before the latter bends mesad to enter the vena jugularis interna.

In its relations and drainage the vena jugularis externa resembles in a general way the like-named vein of the higher vertebrates. On the other hand it seems to correspond to the anterior part of the vena cutanea magna of amphibians. If the latter view is correct, it is not improbable that the vena jugularis externa of higher forms has been derived from the vena cutanea magna of amphibians, the posterior part of the latter vein having been reduced as the cutaneous respiration declined in importance.

B. THE TERRITORY OF THE VENA TRACHEALIS.

The trunk of the vena trachealis has been described by Parker, 84, under the name vena jugularis externa, and by Vogt and Jung, 89-94, p. 714, as "die unpaare Kopfvene," vena cephalica impar. The system of the vena trachealis has been worked out in late embryos of *Lacerta*, in which the larger vessels are presumably in the adult condition. In an embryo which is apparently ready to hatch (head 5.2 mm. long) the vena trachealis (*v. t.*, Text Fig. 3) is an unpaired vein which runs along the right side of the trachea and enters the right vena cava superior directly median to the mouth of the vena jugularis interna. The vena trachealis is formed near the caudal end of the hyoid bone by the union of the right and left tracheal veins. Behind this point the vena trachealis receives no large tributaries, but numerous small veins enter it from the trachea, œsophagus, thymus gland, and the ventral neck muscles. The right and left tracheal veins require a more extended description.

a. VENA TRACHEALIS SINISTRA.

(*v. t. s.*, Text Fig. 3.)

The vena trachealis sinistra begins as a small vein lateral to the body of the hyoid bone. Running directly caudad, the vein passes above the ventral end of the anterior hyoid cornu and receives the vena buccalis sinistra. It then crosses the middle line above the trachea and unites with the vena trachealis dextra.

The *vena buccalis sinistra* drains the floor of the mouth lateral to the tongue, larynx, and the anterior part of the trachea. The rostral part of the vein extends in a nearly sagittal direction, about midway between the tongue and the mandible. Posteriorly the vein bends toward the

middle line to join the vena trachealis sinistra. Lateral to the base of the tongue the vena buccalis forms a connection with the *vena bucco-mandibularis*, which places it in communication with the vena mandibularis externa.

b. VENA TRACHEALIS DEXTRA.

(*v. t. d.*, Text Fig. 3.)

The vena trachealis dextra is formed by the union of the vena buccalis dextra and vena lingualis, which meet above the anterior hyoid cornu, close to the hyoid body. The short trunk runs caudad and meets the vena trachealis sinistra near the caudal end of the hyoid bone.

The *vena buccalis dextra* resembles in every respect the corresponding vein of the other side and requires no special description.

The *vena lingualis* is formed at the base of the tongue, by the union of the venæ linguales, dorsalis and ventralis. The trunk vein runs along the ventral side of the hyoid bone, then bends to the right and joins the vena buccalis dextra, as above described. In front of this junction the vena lingualis receives small tributaries from the larynx, trachea, and hyoglossus muscle. The larger tributaries of the vena lingualis are (1) the *vena lingualis dorsalis* (*v. l. d.*, Text Fig. 3), which drains the tip and the dorsal part of the tongue, (2) *vena lingualis ventralis* (*v. l. v.*, Text Fig. 3), which collects blood from the deeper part of the tongue, the frenulum, and the median part of the floor of the mouth near the symphysis of the lower jaw.

At the rostral end of the hyoid bone the vena lingualis ventralis receives a cutaneous vein, *vena mentalis*, which begins near the symphysis of the jaw and runs backward between the skin and the cerato-mandibular muscles.

c. DEVELOPMENT OF THE VENA TRACHEALIS.

The unsymmetrical arrangement which is shown by the vena trachealis and its tributaries in the adult lizard, is preceded in early embryonic stages by paired venæ tracheales, which discharge, each into the vena jugularis interna of its own side. This condition persists, according to Grosser and Brezina, 95, in an embryo of *Lacerta* with a head 4.1 mm. long, although the left vein is already somewhat smaller than the right. In the ensuing stages a connection is established between the two veins anteriorly and the posterior part of the left vein undergoes degeneration.

II. THE CEPHALIC VEINS AND SINUSES OF TROPIDONOTUS NATRIX.

The literature dealing with the cephalic veins of the Ophidia includes a very few titles. The earliest students of the angiölogy of these forms, such as Schlemm, 27, Jacquart, 55, Nicolai, 26, and Stannius, 56, directed their attention chiefly to the arteries, and the veins were passed over with only casual mention. The first serious work in this field was done by Rathke, 39, who approached the subject from the developmental standpoint; he furnished an excellent account of the large venous trunks of *Tropidonotus*, including the intra-cranial sinuses, which he endeavored to homologize with those of man. Grosser and Brezina, 95, have reviewed Rathke's work and verified many of his conclusions. Through their researches the development of the larger veins has been brought down to the close of embryonic life.

According to Grosser and Brezina, the primitive arrangement of veins described on page 4 undergoes considerable modification in the later embryonic life of *Tropidonotus* (compare Text Fig. 1). By ring formation around the roots of the cranial nerves the vena jugularis interna shifts its position from the ventral to the dorsal side of all nerves, from the trigeminus backward. The vena cerebralis anterior breaks up into two short veins which discharge in opposite directions, the dorsal portion into the vena longitudinalis cerebri, the ventral portion into the jugular vein. The vena cerebralis media forms a new connection (vena cerebralis media secunda, *v. c. m. s.*, Text Figs. 1 and 4) with the vena jugularis interna, and through a secondary connection (*s. V.*, Text Fig. 1) communicates also with the ventral end-piece of the vena cerebralis anterior. In *Tropidonotus* the secondary connection and the ventral end-piece of the vena cerebralis anterior are both intracranial. According to Grosser and Brezina the point of union of the two veins is indicated by their different relations to the ramus ophthalmicus V, the secondary connection lying dorsal, the vena cerebralis anterior ventral, to the nerve.

The following description of the adult relations of the cephalic veins of *Tropidonotus* is intended to supplement the work of Grosser and Brezina:

A. VENA JUGULARIS INTERNA.

(*Vena Capitis Lateralis* Grosser and Brezina.)

The vena jugularis interna of *Tropidonotus* (*v. j. i.*, Text Figs. 1 and 4) arises from the posterior part of the sinus orbitalis, or more definitely,

from a prolongation of the sinus which accompanies the lachrymal gland caudad from the orbit. The vein emerges from the capsule of the gland opposite the anterior end of the prootic bone, where also it receives a musculo-cutaneous tributary from the dorsal region of the head. From the junction of the two veins the vena jugularis interna runs directly caudad, following a course which lies close to the cranial wall and above the roots of the trigeminus and the more posterior cranial nerves. In the anterior part of its course the vein is connected by one or more anastomoses with the posterior prolongation of the sinus orbitalis. Farther caudad it receives two large tributaries from the cranial cavity: the vena cerebrialis media, which passes through the foramen for the second and third branches of the trigeminus, and the vena cerebrialis posterior, which leaves the skull by way of the foramen magnum. Beyond the terminus of the latter vein the vena jugularis interna descends to the side of the œsophagus, where it receives the vena mandibularis, the vena œsophagea, and the vena cervicalis lateralis. The mandibular vein is described below. The vena œsophagea drains the œsophagus and the deeper muscles, the vena cervicalis lateralis (*v. c. l.*, Text Fig. 4) receives blood from the skin and superficial muscles of the neck. These two veins enter the vena jugularis interna at about the same level directly behind the mouth of the vena mandibularis. In Text Fig. 4 the mouth of the vena œsophagea is concealed by the vena jugularis interna.

In this region the jugular vein is enveloped by a small striated muscle, *m. constrictor venæ jugularis internæ*, which also surrounds the terminal parts of the vena mandibularis, vena cervicalis lateralis, and vena œsophagea. The muscle is described in the second part of this paper.

The following tributaries of the vena jugularis interna require a more detailed description:

(a) Sinus orbitalis, (b) vena cerebrialis media, (c) vena mandibularis.

a. THE SINUS ORBITALIS.

(*s. o.*, Text Figs. 1 and 4.)

The sinus orbitalis of the snake is somewhat reduced on account of the absence of movable eyelids; it occupies, however, all of the deeper part of the orbit and reaches laterad somewhat beyond the equator of the bulbus. Anteriorly the sinus extends to the opening of the naso-lachrymal duct; posteriorly it follows the lachrymal gland between the cranial wall and the skin and gives rise to the vena jugularis interna.

The sinus orbitalis is drained largely, perhaps chiefly, by the vena

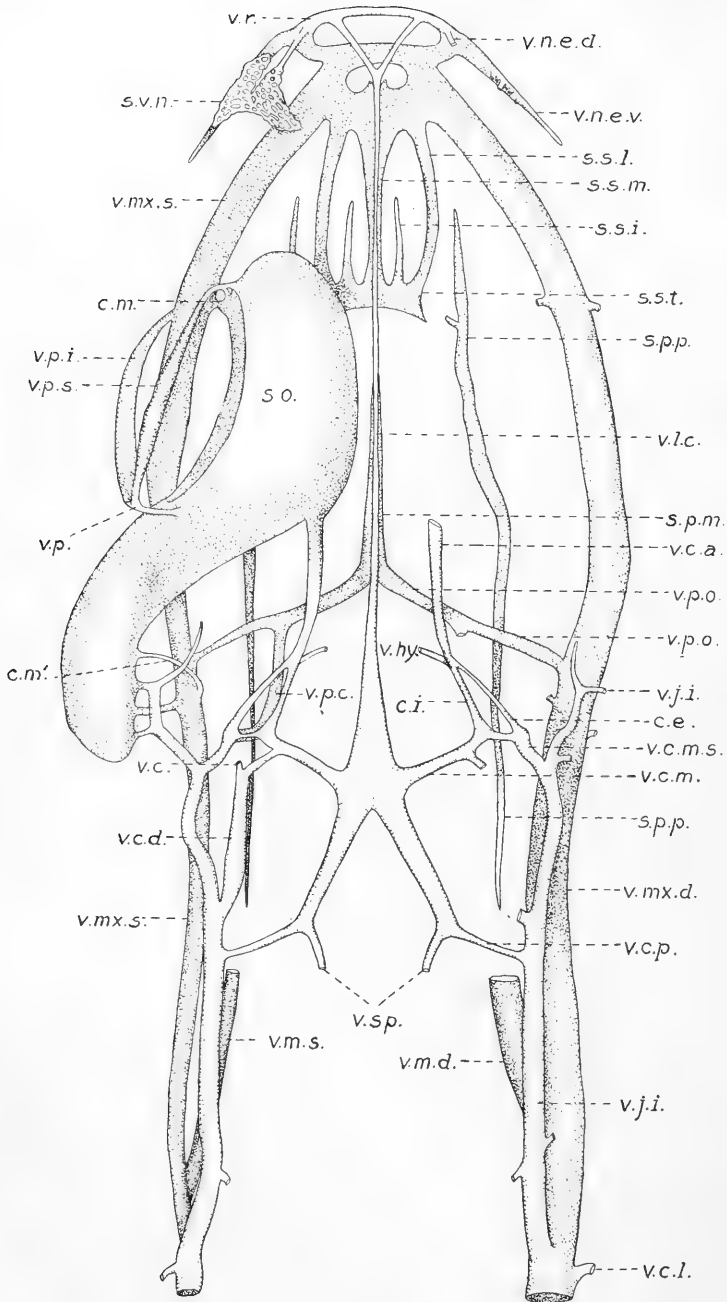


FIG. 4. The cephalic veins of *Tropidonotus natrix*, total length 59 cm. Dorsal view. $\times 9$.

c. e., external secondary anastomosis between the vena cerebralis media and

maxillaris, with which the sinus is connected at different points: (a) Under the anterior part of the bulbus, where sinus and vein come close together, they are connected by a short vertical vein (*c. m.*, Text Fig. 4), which penetrates the floor of the orbit between the maxillary and palatine bones. (b) The posterior prolongation of the sinus, which accompanies the lachrymal gland, communicates with the vena maxillaris by two or more short veins. The first of these (*c. m'*., Text Fig. 4) crosses above the vena maxillaris and joins it on the median side, behind the junction of the vena palato-maxillaris with the maxillary vein. The other veins, varying somewhat in position and number, run from the more posterior part of the sinus orbitalis to the adjacent parts of the vena maxillaris.

The most conspicuous tributary of the sinus orbitalis is the vena palpebralis, which, with its two tributaries, the vena palpebralis inferior and vena palpebralis superior, forms an incomplete ring around the bulbus near the bottom of the cutaneo-palpebral furrow (sinus cutaneo-palpebralis of Ficalbi, 88).

(1) *The vena palpebralis of Tropidonotus* (*v. p.*, Text Fig. 4) is a short vein which is formed in the posterior part of the orbit by the union of the inferior and superior lid veins. The trunk vessel runs caudad a short distance, then bends mesad and discharges into the sinus orbitalis.

(2) *The vena palpebralis inferior* (*v. p. i.*) begins ventral to the posterior end of the lachrymal duct, where, also, the vein communicates with the vena maxillaris. From its origin the vein runs caudad beneath the bulbus, following the margin of the præocular curtain, until it meets the vena palpebralis superior.

(3) *The vena palpebralis superior* (*v. p. s.*) has its origin in the sinus orbitalis under the anterior part of the bulbus, whence it runs dorsad

vena cerebralis anterior; *c. i.*, internal secondary anastomosis between the vena cerebralis media and vena cerebralis anterior; *c. m.*, *c. m'*., anastomoses between sinus orbitalis and vena maxillaris; *s. o.*, sinus orbitalis; *s. p. m.*, sinus palatinus medius; *s. p. p.*, sinus palato-pterygoideus; *s. s. i.*, sinus subnasalis intermedius; *s. s. l.*, sinus subnasalis lateralis; *s. s. m.*, sinus subnasalis medius; *s. s. t.*, sinus subnasalis transversus; *s. v. n.*, sinus vestibuli nasi; *v. c.*, vena cutanea; *v. c. a.*, vena cerebralis anterior; *v. c. d.*, vena capitis dorsalis; *v. c. l.*, vena cervicalis lateralis; *v. c. m.*, vena cerebralis media; *v. c. m. s.*, vena cerebralis media secunda; *v. c. p.*, vena cerebralis posterior; *v. hy.*, vena hypophyseos; *v. j. i.*, vena jugularis interna; *v. l. c.*, vena longitudinalis cerebri; *v. m. d.*, vena mandibularis dextra; *v. m. s.*, vena mandibularis sinistra; *v. mx. d.*, vena maxillaris dextra; *v. mx. s.*, vena maxillaris sinistra; *v. n. e. d.*, vena nasalis externa dorsalis; *v. n. e. v.*, vena nasalis externa ventralis; *v. p.*, vena palpebralis; *v. p. c.*, vena palato-cerebralis; *v. p. i.*, vena palpebralis inferior; *v. p. o.*, vena palatina obliqua; *v. p. s.*, vena palpebralis superior; *v. r.*, vena rostralis; *v. sp.*, vena spinalis.

on the lateral side of the lachrymal duct. Above the front of the bulbus the vein again communicates with the sinus orbitalis, then bends caudad over the bulbus to join the vena palpebralis inferior as stated above. The vena palpebralis and its tributaries are considerably enlarged and form practically a portion of the sinus orbitalis.

A venous ring, surrounding the outer part of the bulbus, was observed by Grosser and Brezina, 95, who derive it from their vena orbitalis superior. Since, however, the ring is not continuous in the adult animal, it has seemed best to reject the name vena orbitalis superior, and to employ the terms used in the description of the palpebral veins of the lizard, to which the veins of the snake correspond, at least in a topographical sense.

b. VENA CEREBRALIS MEDIA.

The primitive relations of the intracranial veins, together with certain changes which occur during late embryonic stages, have been mentioned above. In the case of the vena cerebralis anterior and vena cerebralis posterior I have nothing to add to the account of Grosser and Brezina. The vena cerebralis media, on the other hand, has three anastomoses in the adult snake which are not described by these authors.

(1) In an anterior direction the *vena cerebralis media* shows, in addition to the *internal* "secundäre Verbindung" (*c. i.*, Text Fig. 4), another, *external* anastomosis with the vena cerebralis anterior. The new vein (*c. e.*, Text Fig. 4) begins as an extracranial vessel at the sinus prooticus, from which it runs downward and forward and enters the cranium between the prootic and basisphenoid bones. It joins the vena cerebralis anterior on the lateral aspect of the ramus ophthalmicus V and immediately below the junction of the internal anastomosis with that vein. At the junction of these three veins a vena hypophyseos (*v. hy.*) also forms a connection with the vena cerebralis anterior.

(2) *Vena Palato-cerebralis* (*v. p. c.*, Text Fig. 4).—This vein joins the vena cerebralis media secunda just outside of the foramen for the maxillaris and mandibularis nerves. It places the vena cerebralis media in communication with the system of palatine veins which is described below. Through the vena palato-cerebralis the vena cerebralis media acquires also indirect connection with the vena maxillaris.

(3) *Vena Capitis Dorsalis* (*v. c. d.*, Text Fig. 4).—This sinus-like vein springs from the dorsal portion of the vena cerebralis media, near the junction of the latter with the vena longitudinalis cerebri. It runs dorsad from its origin and escapes from the cranium by a special fora-

men, which lies between the anterior semi-circular canal and the utriculus. Outside of the skull the vein receives a small cutaneous tributary (*v. c.*, Text Fig. 4), then runs directly caudad between the prootic and squamosal bones. Here the vein receives a second cutaneous tributary, then bends directly laterad, under the posterior end of the squamosal bone, and joins the vena jugularis interna.

Through the vena capitis dorsalis indirect connection is established between the vena cerebralis media and the vena jugularis interna. The connecting vein probably forms an important outlet for the escape of blood from the cranial cavity.

The anterior part of the vena capitis dorsalis probably includes the vena cutanea which Grosser and Brezina describe as entering the vena cerebralis media from the top of the head.

c. VENA MANDIBULARIS.

(*v. m.*, Text Figs. 1 and 4.)

The vena mandibularis, as here described, includes the vena maxillaris and vena maxillaris inferior of Grosser and Brezina, whose vena maxillaris superior is called vena maxillaris in this paper. A similar terminology has been generally accepted for the nerves and skeletal parts and is evidently to be preferred also for the veins under consideration.

The vena mandibularis begins in a median sinus which lies behind the ligament connecting the anterior ends of the lower jaw. From the sinus the vein runs caudad between the mandible on one hand and the tongue, larynx, and trachea on the other; it joins the vena jugularis interna under the posterior end of the mandible. At the junction of the two veins a striated constrictor muscle (*m. constrictor venæ jugularis interna*) surrounds both the jugular vein and the terminal portion of the vena mandibularis.

Both venæ mandibulares are considerably enlarged, the right vein (*v. m. d.*, Text Fig. 4) more than the left (*v. m. s.*). Where it joins the vena jugularis interna the right vena mandibularis has about twice the diameter of the vena jugularis itself. On the left side the vena mandibularis and vena jugularis interna are about equal at their junction.

The chief tributary of the vena mandibularis is the vena maxillaris.

VENA MAXILLARIS (*v. mx.*, Text Figs. 1 and 4).—On account of its great length and size the vena maxillaris is the most remarkable vein of the dorsal head region of the snake. The anterior part of the vein is much enlarged and resembles a sinus more than a vein. Near the

rostrum the two venæ maxillares communicate across the middle line of the head, the connection occurring just behind the alveolar portion of the intermaxillary bones, where the veins lie next to the oral mucous membrane. Behind this point the two veins separate a short distance and pass below the nasal cavity. In front of Jacobson's organ they are again connected by a short transverse vein, which corresponds topographically to the sinus palatinus transversus posterior of the lizard. Behind this second anastomosis the two venæ maxillares diverge, each vein running on the dorsal aspect of the interval which separates the maxillary and palatine bones. Under the anterior part of the orbit the vena maxillaris communicates anastomotically with the sinus orbitalis (*c. m.*, Text Fig. 4) and with the vena palpebralis inferior (Text Fig. 4). As the lid vein is connected posteriorly with the sinus orbitalis, it forms a second channel of communication between the sinus and maxillary vein. Near the posterior end of the maxillary bone the vena maxillaris is joined by the vena palato-maxillaris, a vein which aids in forming a connection between the two venæ maxillares across the roof of the mouth. Behind the mouth of the vena palato-maxillaris the vena maxillaris receives an anastomotic vein from the posterior prolongation of the sinus orbitalis, after which it passes under the transverse bone and enters the roof of the mouth. Here the vein continues caudad between the mucous membrane and the *m. pterygoideus externus*. Near the posterior part of the lachrymal gland the vein receives one or more additional anastomoses from the sinus orbitalis. Posteriorly the vena maxillaris meets the vena mandibularis a short distance in front of the junction of that vein with the vena jugularis interna.

Anteriorly the two venæ maxillares of the snake are much enlarged, the right and left veins being about equal. The left vein, however, diminishes in size posteriorly, and as they approach the vena mandibularis the right vein (*v. mx. d.*, Text Fig. 4) has two or three times the diameter of the left (*v. mx. s.*).

Aside from the sinus orbitalis, which has already been described, the most important tributaries of the vena maxillaris are: (1) Vena rostralis, (2) sinus subnasalis, (3) vena subseptalis, (4) vena palatina obliqua.

(1) *Vena Rostralis* (*v. r.*, Text Fig. 4).—The subcutaneous tissue of the rostral region is occupied by a system of small veins which discharge into the vena rostralis. This vein crosses the middle line in front of the intermaxillary bone, then bends caudad on each side and runs on the lateral aspect of the jaw, until it reaches a point ventral to the external

nasal opening. Here the vein bends mesad, under the palatine process of the intermaxillary bone, and discharges into the vena maxillaris lateral to the anterior anastomosis of the two maxillary veins.

The vena rostralis has two important tributaries, the *venæ nasales externæ, dorsalis* and *ventralis*, which run forward, one above, the other below the external nasal opening. The dorsal vein (*v. n. e. d.*, Text Fig. 4) discharges into the dorso-lateral part of the vena rostralis. It has its origin in the fold which forms the median dorsal boundary of the nasal opening. It drains a system of small blood-spaces which surround the anterior part of the duct of the external nasal gland. In a medial direction these blood-spaces occupy the meshes of a small smooth muscle, *m. subnasalis* of the author, 97. Laterally the blood-spaces extend behind the nasal aperture and join a similar system of blood-spaces which lies in the ventral lip of the opening. These ventral blood-spaces discharge for the most part into the vena nasalis externa ventralis (*v. n. e. v.*, Text Fig. 4), a subcutaneous vein which begins a short distance behind the nasal opening and runs forward, under the opening, to discharge into the lateral part of the vena rostralis.

The blood-spaces (*s. v. n.*, Text Fig. 4) drained by the *venæ nasales externæ* represent the sinus vestibuli nasi of the lizard, but this sinus is much reduced in the snake on account of the shortening of the nasal vestibule. The trabeculæ between the blood-spaces contain smooth muscle fibers and the structure is otherwise very similar to that described in the lizard.

In the sea snake, *Hydrophis*, the sinus vestibuli nasi is greatly enlarged (*s. v. n.*, Fig. 4, Plate II), as are all of the veins and sinuses of the anterior part of the head.

(2) *Sinus Subnasalis* (Text Fig. 4).—This sinus lies in the roof of the mouth in front of the choanæ. It discharges into the *venæ maxillares* at their second anastomosis, rostral to the openings of Jacobson's organ. The sinus subnasalis includes (1) a *sinus subnasalis transversus* (*s. s. t.*), a short transverse vessel which lies in the submucosa directly in front of the choanæ; (2) a *sinus subnasalis medius* (*s. s. m.*), which runs in the median line from the sinus subnasalis transversus to the anterior anastomosis of the two *venæ maxillares*; (3) a paired *sinus subnasalis lateralis* (*s. s. l.*), which runs parallel with the last, from the sinus subnasalis transversus to the vena maxillaris. Between the median and lateral sinuses, on each side of the head, lies an opening of Jacobson's organ. In the submucosa behind this opening arises (4) a short *sinus subnasalis intermedius* (*s. s. i.*), which extends caudad and opens into the sinus subnasalis transversus.

Into the sinus subnasalis discharges the *sinus palato-pterygoideus* (*s. p. p.*, Text Fig. 4). This sinus lies on the median side of the palatine and pterygoid bones, beginning at the level of Jacobson's organ and terminating near the level of the foramen magnum. The sinus is much enlarged anteriorly, but it diminishes posteriorly until it is no longer traceable. It reaches its greatest diameter in the neighborhood of the choanæ, behind which it is enclosed in the fold which forms the lateral boundary of the median palatine groove. The sinus discharges through small veins into the sinus subnasalis transversus. Other connections have not been found.

(3) *Vena Subseptalis*.—This irregular, sinus-like vein lies in the connective tissue under the foot of the septum nasale. It has its origin in the submucosa in front of the choanæ and runs forward on the dorsal side of the sinus subnasalis medius. It discharges into the transverse anastomosis which connects the two venæ maxillares in front of the openings of Jacobson's organ.

(4) *Vena Palatina Obliqua* (*v. p. o.*, Text Fig. 4).—Grosser and Brezina observed, 95, in late embryos of *Tropidonotus*, an anastomotic vein which, as described by them, connects the two venæ maxillares across the roof of the mouth just behind the hypophysis. This vein, which is referred to only incidentally, belongs to a system of palatine vessels which must now be described. The system includes a median *sinus palatinus medius* (*s. p. m.*), which begins behind the choanæ and runs caudad through the submucosa, to a point just anterior to the hypophysis. Here the sinus divides to form two *venæ palatine oblique* (*v. p. o.*), which diverge from the median line and run, one on each side of the hypophysis, toward the palato-pterygoid suture. Dorsal to this suture the vena palatina obliqua gives rise to a *vena palato-cerebralis* (*v. p. c.*), which runs dorsad to the foramen for the rami maxillaris and mandibularis V, where it joins the vena cerebralis media just outside of the cranium. Beyond the origin of the vena palato-cerebralis the vena palatina obliqua continues laterad and joins the vena maxillaris.

The veins of the palatine group are more or less enlarged, but unequally so, the paired veins being larger on the right side, corresponding to the inequality of the two venæ maxillares.

III. THE CEPHALIC VEINS OF EMYS EUROPÆA.

The cephalic veins of the Testudinata have not been studied in great detail. The following account, which is based on personal observation, is intended to supplement the descriptions of Bojanus, 19-21, Rathke, 48, and Grosser and Brezina, 95.

A. THE VENA JUGULARIS INTERNA.

The general arrangement of the cephalic veins of *Emys europæa* much resembles that of *Lacerta*, though differing in some important particulars. The vena jugularis interna is a strong vein with definite walls, the anterior part showing no marked enlargement. The vein begins at the caudo-median part of the sinus orbitalis and runs caudad, between the m. retractor oculi and the pterygoid bone, and on the median side of the processus parietalis pterygoidei. This part of the vein lies below the trigeminus nerve and corresponds to the vena cardinalis of Grosser and Brezina. The position of the vein is not intracranial, however, as stated by these authors,² for the vein lies below the eye muscles, while the membranous wall of the cranium lies above these muscles, the conditions being thus identical with those observed in the lizards (compare Figs. 4 and 5, Plate I, and Fig. 1, Plate III).

Caudal to the origin of the trigeminus nerve the vena jugularis interna runs under the tympanic cavity, passing below the auditory and above the post-auditory nerves. Excepting in the region of the trigeminus and auditory nerves the vein corresponds to the vena capitis lateralis of Grosser and Brezina. Under the posterior end of the parotic process the vena jugularis interna receives the vena cerebralis posterior, then bends laterad to meet the vena mandibularis. Beyond its junction with the latter, the vena jugularis interna runs close to the skin and represents a vena jugularis externa (Rathke, 48).

The most important tributaries of the vena jugularis interna of *Emys* are the following:

a. THE SINUS ORBITALIS.

This sinus is well developed in *Emys*, resembling in its distribution the sinus orbitalis of the lizard. In the turtle the sinus is bounded directly by the smooth muscle of the orbit, m. compressor sinus orbitalis, which shows a very strong development (compare page 96). The m. depressor palpebræ inferioris is wanting. The sinus orbitalis receives practically all of the blood from the anterior head region and palate, the chief tributaries being the following:

- (1) *Vena Frontalis*.—This vein drains the region of the frontal bone and discharges into the anterior part of the sinus orbitalis.
- (2) *Sinus Palatinus*.—This sinus is a system of enlarged vessels, which lie close to the oral mucous membrane. It includes (a) a *sinus*

² Gaupp, *oo*, p. 548, has pointed out this error.

palatinus medius, which begins at the rostrum and extends caudad, in the middle line, to a point just behind the choanæ. In front of the choanæ the sinus receives, on each side, a vein from the median wall of the nasal cavity. Behind the choanæ the sinus palatinus medius divides into two branches, which diverge from the middle line and discharge into (b) the *sinus palatinus lateralis*. This sinus begins behind the dentary portion of the intermaxillary bones, where the two sinus palatini laterales and the sinus palatinus medius meet in a common anastomosis. Each sinus palatinus lateralis extends caudad under the posterior part of the sinus orbitalis, into which it discharges through a short vessel which lies median to the suture connecting the palatine parts of the maxillary and jugal bones.

Near its posterior end the sinus palatinus lateralis receives the *vena maxillaris*. This is for the most part an intraosseous vein which is enclosed in the same bony canal with the arteria maxillaris and the ramus maxillaris V. Approaching the posterior end of the maxillary bone the vein and nerve bend mesad, emerge from the bone and enter the large foramen suborbitalis, through which the vein bends ventrad to join the sinus palatinus lateralis.

b. VENA CEREBRALIS MEDIA.

This vein leaves the cranial cavity through the trigeminal foramen, on the outside of which it runs downward and forward to join the vena jugularis interna. The extracranial portion of the vein corresponds apparently to the vena cerebialis media secunda of Grosser and Brezina. In *Emys* this vein is connected with the sinus orbitalis by a vein of considerable size, which lies in the angle between the cranial wall and the pterygoid process of the parietal bone. The vein lies on the lateral aspect of the ramus ophthalmicus V, and both vein and nerve are extracranial. This vein includes presumably, the secondary connection of the vena cerebialis media and the ventral end-piece of the vena cerebialis anterior.

c. VENA CEREBRALIS POSTERIOR.

This vein, which is the chief efferent vessel of the brain in *Emys*, arises from the posterior end of the vena longitudinalis cerebri and leaves the cranium in two parts, one of which passes through the foramen magnum, the other through the foramen jugulare. Outside of the skull the two branches of the vein unite, the trunk vein running laterad to join the vena jugularis interna under the parotic process. The ring formed

by the vena cerebialis posterior in *Emys* resembles the temporary ring observed by Grosser and Brezina in their *Lacerta* embryo, series XIV, head 4.1 mm.

d. VENA MANDIBULARIS.

This vein has not been fully worked out. Its posterior part lies lateral to the mandible, where it receives a small vein from the side of the head. The vena mandibularis discharges into the posterior end of the vena jugularis interna, near the junction of the latter with the vena jugularis externa.

IV. GENERAL REMARKS AND SUMMARY ON THE CEPHALIC VEINS OF THE SAURIA, OPHIDIA, AND TESTUDINATA.

1. In *Lacerta* and *Emys* practically all of the blood of the anterior part of the head is discharged into the sinus orbitalis, which includes among its tributaries the vena maxillaris (v. maxillaris superior of authors). In *Tropidonotus* the blood of the anterior region of the head passes partly into the sinus orbitalis, partly into the vena maxillaris which has direct connection with the vena mandibularis behind the orbit.

2. In *Lacerta* and *Emys* the sinus orbitalis is drained only by the vena jugularis interna. In *Tropidonotus* the vena jugularis interna arises from the sinus orbitalis but the anterior part of the vein is small and carries little blood from the anterior part of the head. The chief outlet of the sinus orbitalis of the snake is the vena maxillaris.

3. In all of the forms studied, lizard, snake, and turtle, the vena jugularis interna eventually receives the greater part of the blood from the head. In the lizard, under ordinary conditions, the vein probably carries nine-tenths of all the blood from the face and cranium, the remainder passing through the vena jugularis externa, vena spinalis, and smaller vessels.

4. The veins of the brain undergo little modification with the approach of adult life. In *Tropidonotus* and *Emys* the ventral end-piece of the vena cerebialis anterior and the secondary connection of the vena cerebialis media form a continuous vein which runs from the sinus orbitalis to the vena cerebialis media. In the snake this vein is intracranial; in the turtle it is extracranial. In *Lacerta* a similar extracranial vein occurs but it is much reduced, owing to the posterior extension of the sinus orbitalis; it has little importance in the adult lizard.

The vena cerebialis media of *Tropidonotus* shows the following anastomoses: (a) With the vena jugularis interna, through the vena capitis

dorsalis; (*b*) with the palatine veins, through the vena palato-cerebralis; (*c*) an external connection with the vena cerebralis anterior; (*d*) an internal connection (secondäre Verbindung) with the vena cerebralis anterior.

In *Lacerta* the vena cerebralis media sometimes retains its posterior outlet into the vena jugularis interna.

The vena cerebralis posterior gives rise in all forms, to a vena spinalis, which may, perhaps, furnish an outlet for the blood of the brain.

5. All of the forms studied show an extensive system of palatine sinuses or sinus-like veins. In *Lacerta* and *Emys* these include certain apparently homologous parts, especially the sinus palatinus medius and sinus palatinus lateralis. In the snake the relations of the various parts are peculiar and difficult to interpret. Some of these relations are referred to in connection with the vena maxillaris.

6. The vena mandibularis of *Lacerta*, *Tropidonotus*, and *Emys* enters the vena jugularis interna near the posterior end of the mandible. In the lizard and snake the junction of the two veins occurs at a point where the trunk vein is surrounded by a striated constrictor muscle, *m. constrictor venæ jugularis internæ*, which is described in the second part of this paper. This relation seems to furnish conclusive evidence that the vena mandibularis of the snake is homologous with the vena mandibularis of the lizard.

In the turtle the vena mandibularis is a small vein which enters the jugular vein caudal to the constrictor muscle. It is not improbable that the terminal part of the mandibular vein has been utilized by the vena jugularis interna in order to make connection with the vena jugularis externa. This would also explain the lateral termination of the mandibular vein in the turtle.

In *Tropidonotus* the vena mandibularis is considerably enlarged, the right vein more than the left. Its chief tributary is the vena maxillaris. In the *Sauria* the vena mandibularis is formed by the union of two veins, the external and internal mandibular veins, which are connected by numerous anastomoses. The vena maxillaris of the lizard terminates in the sinus orbitalis and has no connection with the postorbital veins.

7. The vena maxillaris shows a similar development in the lizard and turtle. It would probably be impossible, however, to establish complete homology between the saurian vein and the vena maxillaris of the snake. On the contrary, if we compare the anterior part of the latter vein with the sinus palatinus of the lizard, we find certain resemblances which do not seem to be accidental. The sinus-like enlargement, the relation to

the oral mucous membrane and to the upper jaw, and the two anastomoses, one behind the rostrum, the other in front of Jacobson's organ, are common to both vessels. It seems probable, therefore, that the anterior part of the vena maxillaris of the snake has been derived from the sinus palatinus lateralis of saurian ancestors. The anterior part of the vena maxillaris of the lizard seems to be represented in the snake by the vena nasalis externa ventralis, whose relation to the vena rostralis may be explained by obliteration of the posterior part of the maxillary vein.

The orbital part of the vena maxillaris of the snake might be derived either by median migration of the vena maxillaris of the lizard or by a dorsal migration of the sinus palatinus lateralis. The postorbital part of the vena maxillaris of the lizard has probably been obliterated.

8. The cephalic veins of lizards, snakes, and turtles are connected by numerous anastomoses, especially in the adult stage. These anastomoses are for the most part open passages, permitting the movement of blood in either direction. They apparently serve to equalize the blood-pressure in different parts of the head.

9. The most striking characteristic of the venous system of the Sauria, Ophidia, and Testudinata is the abundance of blood sinuses in the head. These include not only intracranial sinuses, similar to those of other vertebrates, but also extracranial sinuses which probably do not occur elsewhere in the vertebrate series. The significance of these extracranial sinuses will be considered in the second part of this paper.

PART SECOND.

ON THE SIGNIFICANCE OF THE CEPHALIC SINUSES OF THE SAURIA, OPHIDIA, AND TESTUDINATA.

The extraordinary development of blood sinuses in the head of the Sauria, Ophidia, and Testudinata suggests a lacunar blood system, such as we find in many invertebrate animals. The resemblance is a superficial one, however, for the reptilian sinuses are formed during embryonic life by the enlargement of veins and capillaries. Moreover, they are limited in their distribution to the cephalic region and, therefore, their existence must be explained by a study of local conditions.

An investigation of the cephalic sinuses of the Reptilia was suggested several years ago by certain preliminary studies on the ejection of blood by *Phrynosoma*. In order to explain this phenomenon it seemed necessary to assume a temporary increase of blood-pressure in the region of

the eye. A thorough examination by means of sections led to the discovery of a special muscle for the obstruction of the vena jugularis interna. This observation promised at least a partial solution of the problem. Further study, however, soon revealed the fact that the same muscle occurs in other Sauria, and afterwards it was found also in the Ophidia and Testudinata.

These discoveries diverted my attention from Phrynosoma and led to a study of the wider significance of the peculiar mechanism. As a first step I undertook an investigation of the cephalic veins and sinuses and their relation to other organs of the head. In the course of this investigation it was found that the muscle for obstructing the vena jugularis interna is everywhere associated with enlarged cephalic veins and sinuses. In the Sauria other muscles are also discovered which assist in raising the blood-pressure in the distended vessels. It was, however, not to be supposed that this *swell mechanism* is extensively used for the ejection of blood. Its wide distribution pointed rather to the existence of an undiscovered function of fundamental importance in the life of its possessors. A function which seems to meet the demands of the case was finally observed in the Sauria.

In the following account of the swell mechanism I shall begin with the Sauria, which have been more thoroughly studied than the Ophidia and Testudinata. I shall describe first of all the mechanism for obstructing the vena jugularis interna and raising the blood-pressure in the veins and sinuses of the head.

I. DESCRIPTION OF A SWELL MECHANISM IN THE HEAD OF SAURIA.

A. MUSCLES WHICH OBSTRUCT THE VENA JUGULARIS INTERNA AND RAISE THE VENOUS BLOOD-PRESSURE IN THE HEAD OF THE SAURIA.

In the Sauria as a group the mechanism for raising the blood-pressure in the veins and sinuses of the head includes three muscles which must first of all receive adequate description. These muscles I designate as follows:

- a. *Musculus constrictor venæ jugularis internæ.*
- b. *Musculus protrusor oculi.*
- c. *Musculus protrusor oculi accessorius.*

a. THE MUSCULUS CONSTRICTOR VENÆ JUGULARIS INTERNÆ.

1. ANATOMICAL RELATIONS.—The *m. constrictor venæ jugularis internæ*, which was first described by the writer of this article, 98, is a

striated muscle which surrounds the jugular vein in the region where the latter passes from the head into the neck. In its simpler form the muscle has a single attachment to the skeletal parts, a portion of its fibers arising from the parotic process³ directly lateral to the vein. This form of the constrictor muscle occurs in *Phrynosoma* and *Monitor*.

In *Phrynosoma cornutum* the constrictor muscle (Text Fig. 5) envelops the vena jugularis interna for a distance of about 1600 μ . The attachment of the muscle begins about 400 μ behind the anterior end

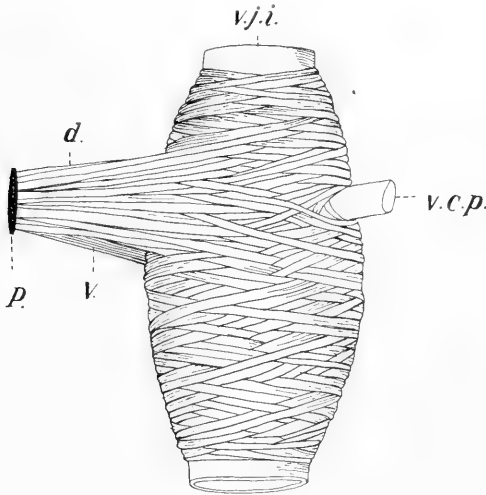


FIG. 5. *M. constrictor venæ jugularis internæ* of *Phrynosoma cornutum*, left side, from above. $\times 32$.

The general relations were obtained by reconstruction. The muscle fibers are drawn to scale but their arrangement is somewhat diagrammatic.

P., part of the parotic process; *v. j. i.*, vena jugularis interna; *v. c. p.*, vena cerebralis posterior.

and continues caudad 450 μ . Opposite from the attachment of the muscle the vena cerebralis posterior (*v. c. p.*) penetrates the muscle to reach the jugular vein (compare also Figs. 1 and 2, Plate I).

The fibers of the muscle spring from the posterior descending part of the parotic process, partly from the occipitale laterale (*Ol.*, Fig. 2, Plate I), partly from a remnant of cartilage which lies between the

³This is the parotic process of Parker, 84, p. 140, which is formed in the adult chiefly by the occipitale laterale and the opisthoticum. In the embryo it is composed of cartilage, some of which may persist in the adult.

occipitale laterale and the supratemporale. From their origin the fibers of the muscle extend mesad in two fan-shaped bundles, one above, the other below the vein, which the fibers closely invest in a spiral direction. These fibers terminate in the wall of the vein or in the surrounding con-

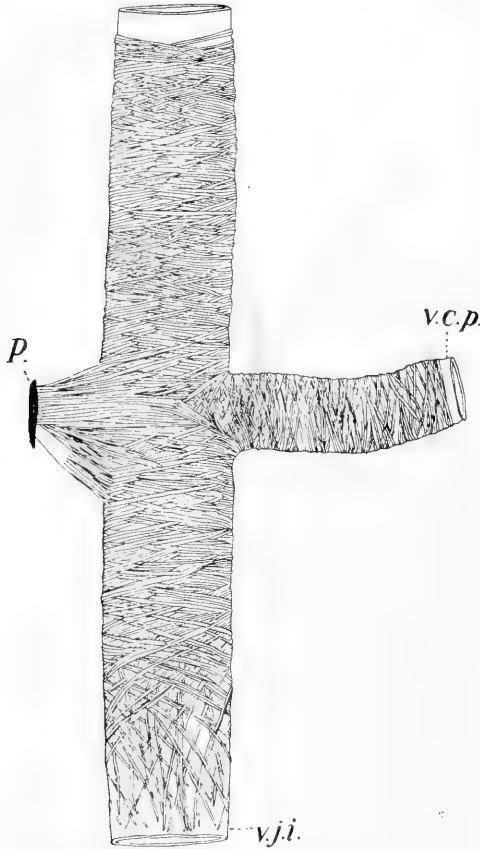


FIG. 6. *M. constrictor venæ jugularis internæ* of *Monitor niloticus*, left side, from above. $\times 32$.

The general relations were determined by reconstruction. The muscle fibers are drawn to scale but their arrangement is somewhat diagrammatic.

P., part of parotic process; *v. j. i.*, vena jugularis interna; *v. c. p.*, vena cerebral posterior.

nective tissue. Mixed with these there are other spiral fibers which both begin and end in the wall of the vein. The middle portion of the muscle includes altogether seven or eight layers of these spiral fibers, partly free, partly attached. Toward the ends of the muscle the number of layers

is gradually reduced. Inside of the spiral fibers the muscle contains one or two layers of longitudinal fibers which are deeply imbedded in the wall of the vein.

In *Monitor niloticus* (Text Fig. 6) the relations of the m. constrictor venæ jugularis internæ are the same as in *Phrynosoma*. In a specimen 28 cm. long the muscle covers the vein for a distance of 3.2 mm. The fibers of the muscle form a close network not only around the vena jugularis interna but also about the vena cerebialis posterior, which they follow half way to the foramen magnum. The muscle fibers themselves are relatively slender, as are all of the striated fibers in *Monitor*.

In *Lacerta* the relations of the m. constrictor venæ jugularis internæ are complicated by the attachment of the muscle to a free epibranchial cartilage,⁴ the second epibranchial of Parker, 84. This cartilage is an irregularly curved rod which extends from the roof of the tympanum to the floor of the pharynx. It includes (a) an oblique anterior portion, which arises from the lateral wall of the prominentia ampullæ posterioris; (b) a middle, sagittal portion, which lies on the median aspect of the m. constrictor venæ jugularis internæ; it is provided with a strong dorsal ridge for the attachment of the muscle (Text Fig. 7; Fig. 3, Plate I). (c) The caudal portion of the cartilage bends under the jugular vein and around the lateral wall of the pharynx, where it lies caudal to the first cerato-branchial. It terminates in the floor of the pharynx a short distance behind the caudal end of the second cerato-branchial cartilage.

The m. constrictor venæ jugularis internæ of *Lacerta agilis* surrounds the vein at the terminus of the vena mandibularis and vena cerebialis posterior (Text Figs. 2 and 7). In a specimen 18 cm. long the muscle envelops the vein for a distance of about 2 mm., one-half of which lies behind the mouth of the vena cerebialis posterior. The vena mandibularis enters the jugular vein anterior to the mouth of the cerebral vein (Text Figs. 2 and 7). The lateral attachment of the muscle lies wholly rostral to the mouth of the vena mandibularis; it begins about 260 μ behind the anterior border of the muscle and covers 240 μ . The median attachment of the muscle begins about 540 μ behind the anterior border and includes about 500 μ , or one-fourth of the total length of the muscle.

The constrictor muscle is composed of both free and fixed fibers. In the cartilaginous stage of the skull the fixed fibers arise wholly from the lateral portion of the parotic process (crista parotica of Gaupp, oo).

⁴Cope, 98, has also observed this cartilage in *Lacerta* and noted its close approximation to the second cerato-branchial cartilage.

In later stages, when this part of the parotic process ossifies to form the lateral extremity of the occipitale laterale, the majority of the muscle fibers are attached to that bone (*Ol.*, Fig. 3, Plate I), while the remainder arise from a membrane bone, the supra-temporale (*St.*), which covers the lateral part of the parotic process. From their origin the muscle fibers extend mesad and somewhat caudad, partly above, partly below the vena jugularis interna. The larger number of these fibers, both dorsal and ventral, insert on the crista epibranchialis (*C. ep.*, Text Fig. 7; Fig. 3, Plate I); the remaining fibers envelope the vein, the anterior fibers coiling forward, the posterior fibers backward, from their origin. The free fibers of the muscle are either spiral or longitudinal in direction, the spiral fibers being mixed with those which spring from the parotic process, while the longitudinal fibers lie for the most part inside of the spiral fibers next to the lumen of the vein.

The stronger anterior part of the muscle includes a total of four or five layers of fibers, which form a close covering for the vein as far caudad as the mouth of the vena cerebralis posterior. Behind this point the muscle is less compact and contains a large proportion of longitudinal fibers. On the whole the constrictor muscle is not so strongly developed in *Lacerta* as in the other forms already mentioned.

In addition to the species referred to, the m. constrictor venæ jugularis internæ has been anatomically demonstrated in the following forms:

- Agama colonorum Daudin.
- Moloch horridus Gray.
- Uta stansburiana Baird and Girard.
- Anolis caroliniensis Cuvier.
- Sceloporus undulatus Latreille.
- Anguis fragilis Linnaeus.
- Lacerta viridis Linnaeus.
- Lacerta muralis Merr.
- Cnemidophorus sexlineatus Linnaeus.

In all of these forms the constrictor muscle is well developed. It arises in all cases from the parotic process, chiefly from the ossified crista parotica (occipitale laterale), and when the second epibranchial cartilage is present, the median part of the muscle is attached to it. I have found this attachment in the Teiidae (*Cnemidophorus*) and Iguanidae (*Sceloporus*), as well as in the Lacertidae.⁵

⁵ According to Cope, 98, free epibranchials occur also in the Scincidae and Xantusiidae, but no representatives of these families have been studied.

In *Phrynosoma* and in all forms in which the muscle has a single attachment, the *m. constrictor venæ jugularis internæ* acts merely as a constrictor of the jugular vein. In *Lacerta*, on the other hand, the muscle not only obstructs the vein but also changes the position of the epibranchial cartilage, which is moved through a small arc about its fixed anterior end. This fact explains the oblique direction of the fibers which insert on the cartilage (compare Text Fig. 7), their course being parallel with the arc described by the *crista epibranchialis*. This second function does not seem to impair the efficiency of the muscle as a constrictor, for the fibers which insert on the epibranchial cartilage form a closed ring, and owing to the movability of the cartilage, they still act as an effective constrictor of the vein.

2. INNERVATION OF THE *M. CONSTRICTOR VENÆ JUGULARIS INTERNÆ*.

—Before describing the innervation of the *m. constrictor venæ jugularis internæ* of *Lacerta*, I must call attention to the following nerves which lie in close proximity to the muscle:

(1) *Ramus communicans internus n. glossopharyngei cum n. faciali* (*ramus communicans internus rami palatini cum n. glossopharyngeo* of Fischer, 52).—This nerve (*r. c. i.*, Text Figs. 2 and 7; Fig. 3; Plate I) arises from the ganglion glossopharyngei (ganglion petrosum of authors) and runs forward on the median side of the *vena jugularis interna*. It passes under or through the median attached portion of the constrictor muscle, then between the epibranchial cartilage and the prætympanic furrow, and eventually joins the *ramus palatinus VII* near its origin from the ganglion facialis.

(2) *Ramus communicans externus n. glossopharyngei cum n. faciali* (*ramus communicans externus n. facialis cum n. glossopharyngeo* of Fischer, 52).—This nerve (*r. c. e.*, Text Figs. 2 and 7; Fig. 3, Plate I) begins at the ganglion glossopharyngei and runs forward on the dorsal aspect of the *ramus internus*. It passes through or immediately above the median attached portion of the constrictor muscle, anterior to which it bends laterad under the jugular vein and enters the sheath of the *ramus posterior VII*. A part of its fibers join the latter nerve, but not all; about one-half of the fibers emerge from the nerve laterally and join the *ramus communicans n. glossopharyngei cum n. maxillari*, as described below.

The *rami communicantes n. glossopharyngei cum n. faciali* are sometimes united for a short distance anterior to the ganglion glossopharyngei.

(3) *Ramus communicans n. glossopharyngei cum n. maxillari.*— This nerve includes the ramus recurrens n. maxillaris ad n. facialem of Fischer, 52, and Watkinson, 06, which, as described by these authors, runs from the ramus maxillaris V over the top of the head to the ramus posterior VII. A study of *Lacerta* and *Monitor* has shown, however, that Fisher's nerve is really but a part of a longer tract which connects the glossopharyngeus with the ramus maxillaris V. This nerve,

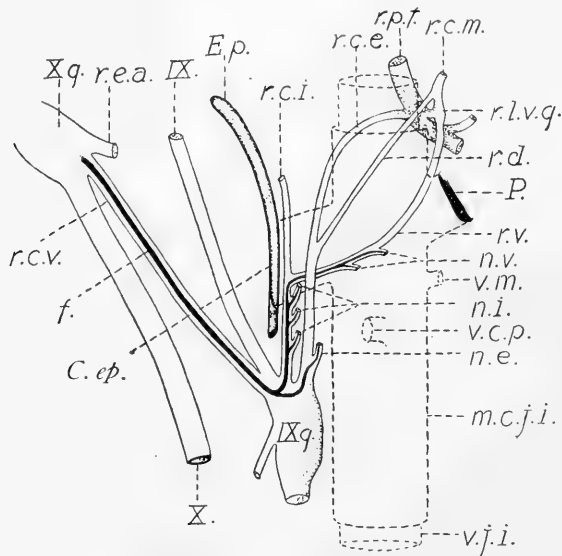


FIG. 7. Diagram showing the origin of the nerve fibers and nerves which supply the m. constrictor venae jugularis internae in *Lacerta agilis*. Compare also Text Fig. 2.

Ep., second epibranchial cartilage of Parker, with crista epibranchialis (*C.ep.*); *f.*, bundle of nerve fibers which form the nervi tumefactores; *m. c. j. i.*, the dotted line shows the outline of the m. constrictor venae jugularis internae, whose attachments are at *P.* (parotic process) and *C. ep.* (crista epibranchialis); *n. e.*, *n. i.*, *n. v.*, nervi tumefactores arising respectively from the ramus communicans externus n. glossopharyngei cum n. faciali, from the ramus communicans internus n. glossopharyngei cum n. faciali and from the pars ventralis of the ramus communicans n. glossopharyngei cum n. maxillari; *P.*, parotic process; *r. c. m.*, ramus communicans n. glossopharyngei cum n. maxillari; *r. c. v.*, ramus communicans n. vagi cum n. glossopharyngeo; *r. d.*, pars dorsalis of the ramus communicans n. glossopharyngei cum n. maxillari; *r. c. e.*, ramus communicans externus n. glossopharyngei cum n. faciali; *r. e. a.*, ramus externus accessorii; *r. c. i.*, ramus communicans internus n. glossopharyngei cum n. faciali; *r. l. v. g.*, ganglion at junction of the pars ventralis and pars lateralis of the ramus communicans n. glossopharyngei cum n. maxillari; *r. p. f.*, ramus posterior facialis; *r. v.*, pars ventralis of the ramus communicans n. glossopharyngei cum n. maxillari; *v. c. p.*, vena cerebialis posterior; *v. j. i.*, vena jugularis interna, represented by dotted line; *v. m.*, vena mandibularis; *IX, X*, cranial nerves; *Xg.*, ganglion superius vagi; *IXg.*, ganglion glossopharyngei.

which I shall call *ramus communicans n. glossopharyngei cum n. maxillari*, arises in *Lacerta agilis* by three roots, which may be distinguished as dorsal, ventral, and lateral.

The dorsal root, *pars dorsalis* (*r. d.*, Text Figs. 2 and 7), is united at its origin with the *ramus communicans externus n. glossopharyngei cum n. faciali*. The common trunk arises from the ganglion *glossopharyngei* and runs forward as far as the median attachment of the constrictor muscle. Here the two nerves separate, the *pars dorsalis* running laterad above the jugular vein to join the *pars ventralis* and the *pars lateralis*, as described below, while the *ramus communicans externus* passes below the vein to reach the *ramus posterior VII*.

The ventral root, *pars ventralis* (*r. v.*, Text Figs. 2 and 7; Fig. 3, Plate I). The fibers of this root usually leave the ganglion *glossopharyngei* with the *ramus communicans internus n. glossopharyngei cum n. faciali*. At the median attachment of the constrictor muscle the *pars ventralis* fibers leave the common trunk and form a separate nerve, which bends laterad through the ventral part of the constrictor muscle. On the lateral aspect of the jugular vein, and just in front of the lateral attachment of the constrictor muscle, the *pars ventralis* joins the *pars lateralis*.

In one of the specimens examined the *pars ventralis* is a separate nerve from its origin at the ganglion *glossopharyngei* to its junction with the *pars lateralis*.

The lateral root, *pars lateralis*. This nerve seems to spring from the *ramus posterior facialis* (Text Figs. 2 and 7). A careful examination has shown, however, that it comes from the *ramus communicans externus n. glossopharyngei cum n. faciali*, the fibers of the *pars lateralis* passing directly through the dorsal part of the *ramus posterior VII* and emerging on the lateral aspect of that nerve, where they join the *pars ventralis*. The junction of the two nerves is marked by a small ganglion (*r. l. v. g.*, Text Fig. 7), from which a single nerve turns dorsad and join the *pars dorsalis*, thus forming the trunk of the *ramus communicans n. glossopharyngei cum n. maxillari*. This nerve enters a groove on the rostral surface of the parotic process and ascends to the top of the head, where it runs forward in company with the *vena supratemporalis* and the *arteria temporo-muscularis* (*r. c. m.*, Text Figs. 2 and 7; Fig. 4, Plate I).

The three nerves just described (*rami communicantes, internus and externus, n. glossopharyngei cum n. faciali and ramus communicans n. glossopharyngei cum n. maxillari*) occur also in *Monitor*, in which their relations are more easily demonstrated than in *Lacerta*. The *rami*

communicantes n. glossopharyngei cum n. faciali begin as separate nerves at the ganglion glossopharyngei, though they may be connected by anastomoses after they leave the ganglion. In front of their origin the ramus internus accompanies the glossopharyngeus and has no direct connection with the ramus communicans n. glossopharyngei cum n. maxillari. The ramus communicans externus n. glossopharyngei cum n. faciali passes under the constrictor muscle and approaches the ramus posterior VII at a point median to the origin of the chorda tympani. Here the ramus externus divides into three branches, one of which bends caudad and enters the peripheral part of the ramus posterior VII, while a second branch bends forward and joins the proximal part of the same nerve. The third nerve crosses above the ramus posterior VII and divides into two parts. One of these joins the chorda tympani, the other is the pars lateralis of the ramus communicans n. glossopharyngei cum n. maxillari. Near its origin the pars lateralis is provided with a small ganglion, from which the nerve runs dorsad to join the pars dorsalis.

The ramus communicans n. glossopharyngei cum n. maxillari has but two roots in Monitor. The dorsal root, pars dorsalis, arises as a separate nerve from the ganglion glossopharyngei or from the cervical sympathetic close to that ganglion. It runs forward on the median side of the vena jugularis interna until it reaches a point just caudal to the vena cerebralis posterior, where the nerve bends laterad above the jugular vein. It unites with the pars lateralis in front of the lateral attachment of the constrictor muscle.

The m. constrictor venæ jugularis internæ of *Lacerta agilis* is innervated by small nerves which I designate *nervi tumefactores capitis* (see Text Fig. 7). In *Lacerta* they include:

(a) From one to three nerves (*n. v.*), which spring from the pars ventralis of the ramus communicans n. glossopharyngeus n. maxillari. They emerge from the common trunk as it passes through the ventral part of the constrictor muscle; they supply the ventral and lateral parts of the muscle.

(b) From two to four nerves (*n. i.*), which arise from the ramus communicans internus n. glossopharyngei cum n. faciali as that nerve approaches the median attachment of the constrictor muscle. These nerves enter the median attached part of the muscle and separate, some supplying the dorsal, some the ventral part of the muscle.

(c) One nerve (*n. e.*) from the ramus communicans externus n. glossopharyngei cum n. faciali. It arises from the latter nerve a short distance in front of the ganglion glossopharyngei and runs directly forward

into the median part of the constrictor muscle. It supplies the dorsal fibers of the muscle.

The number and origin of the *nervi tumefactores capitis* are variable in different specimens and even on opposite sides of the head in the same individual. In one specimen the nerves of one side seem to spring wholly from the *pars ventralis* of the *ramus communicans n. glossopharyngei cum n. maxillari*. In the same specimen on the other side of the head, the nerves arise partly from the *pars ventralis*, partly from the *ramus communicans internus n. glossopharyngei cum n. faciali*. In a second individual the tumefactor nerves spring from the *pars ventralis* and from the *ramus communicans externus n. glossopharyngei cum n. faciali*. Other variations would doubtless be found in other specimens.

The origin and path of the tumefactor nerves, as described above, has been determined entirely by a study of sections. The course of the fibers which compose the nerves has been further worked out experimentally as follows (compare Fig. 7) :

(a) The fibers of the tumefactor nerves come from a posterior direction and not from the anterior part of the *ramus communicans n. glossopharyngei cum n. maxillari*. Proof of this fact was furnished both by the microscope and by experiment. Cutting of the *ramus communicans n. glossopharyngei cum n. maxillari* on top of the head in the living animal did not interfere with the natural contraction of the constrictor muscle one or two hours after the operation. Stimulation of the posterior end of the cut nerve had no effect on the muscle.

(b) The fibers of the *nervi tumefactores* pass through the ganglion *glossopharyngei*. In order to obtain positive evidence on this point a specimen of *Lacerta muralis* was etherized, parts of the skin and superficial muscles (*cucullaris*, *capiti-mandibularis*, *episterno-cleido-mastoi-deus*) were removed so as to expose the constrictor muscle and the adjacent cranial nerves. Stimulation of the ganglion *glossopharyngei* (*IXg.*, Text Fig. 7) under a lens resulted in strong contraction of the *m. constrictor venæ jugularis internæ*.

(c) The fibers which supply the constrictor muscle come from the ganglion *radicis vagi* (ganglion *superius vagi* of authors). Negative results were obtained by stimulating both the *glossopharyngeus* and the *hypoglossus*. Stimulation of the ganglion *radicis vagi* (*Xg.*, Text Fig. 7), on the other hand, gave contraction of the constrictor muscle. From this ganglion the fibers of the tumefactor nerves pass neither through the *vagus* nor *accessorius*, but through the *ramus communicans n. vagi*

cum n. glossopharyngeo,⁶ a short nerve (*r. c. v.*, Text Figs. 2 and 7), which runs directly from the ganglion radiceis vagi to the ganglion glossopharyngei. Direct stimulation of this nerve caused contraction of the constrictor muscle. Stimulation of the ganglion radiceis vagi after cutting this nerve gave a negative result.

These different tests were repeated with different specimens, both of *Lacerta muralis* and *Sceloporus undulatus*. They show that the fibers which innervate the constrictor muscle must be referred back to the ganglion radiceis vagi, whence they run through the ramus communicans n. vagi cum n. glossopharyngeo to the ganglion glossopharyngei. From this ganglion, in *Lacerta agilis*, the fibers continue forward, usually in a single bundle, which passes through the ramus communicans internus n. glossopharyngei cum n. faciali. From the latter the tumefactor fibers pass to the constrictor muscle either directly or through the pars ventralis of the ramus communicans n. glossopharyngei cum n. maxillari. In some cases the fibers of the tumefactor nerves leave the ganglion glossopharyngei in two bundles, one of which follows the path just described, while the other enters the ramus communicans externus n. glossopharyngei cum n. faciali.

In the course of these experiments on *Lacerta* and *Sceloporus* it was observed that the constrictor muscle could be stimulated in a reflex way through the spinal nerves of the neck. The passage of the impulse through the ganglion radiceis vagi was indicated by division of the ramus communicans n. vagi cum n. glossopharyngeo, after which stimulation of the spinal nerves gave no response. An apparently reflex contraction of the constrictor muscle has also been observed after stimulation of sensory nerves in various parts of the head. It is not improbable, therefore, that under natural conditions the contraction of the constrictor muscle may be a more or less reflex act due to stimulation of sensory nerves.

I do not attempt to determine whether the nervi tumefactores capitis belong to the vagus or to the accessorius nerve trunk, as in the present state of our knowledge it is impossible to draw a line between these nerves. The following facts, however, seem to indicate an origin from the so-called vagus portion of the vago-accessorius complex:

In *Cistudo carolina* the fibers which innervate the constrictor muscle come from the anterior roots of the vago-accessorius series—roots which

⁶ According to Hoffmann, 90, page 745, the ramus communicans nervi vagi cum n. glossopharyngeo also contains the fibers of the n. laryngeus superior

are commonly considered as belonging to the vagus. It is very probable that a similar relation exists in other forms in which the constrictor muscle occurs.

In the lizard the motor fibers of the tunefactor nerves are relatively small, resembling those of the vagus trunk, while the chief motor branch of the accessorius (ramus externus accessorii) is composed of very large fibers.

The relations of the constrictor muscle, especially its attachment to the second epibranchial cartilage, suggests its derivation from a branchial muscle of the second arch, which in lower forms is supplied by the vagus nerve.

3. ONTOGENY AND PHYLOGENY OF THE M. CONSTRICTOR VENÆ JUGULARIS INTERNÆ.—The embryological development of the constrictor muscle begins a little later than that of the other striated muscles of the head. In a *Lacerta* embryo with head 1700 μ long no trace of the constrictor fibers could be seen, although the fibers of other muscles had begun their development. In *Sceloporus* the formation of the primitive fibers begins with a head-length of about 1900 μ . In a specimen with the head 2100 μ long the development of the fibrillæ in the periphery of the fibers has begun. In a later stage, head 2500 μ long, the number of fibers has considerably increased and the development is well advanced. In both *Sceloporus* and *Lacerta* the muscle fibers are fully formed at the time of hatching.

According to the above account the fibers of the m. constrictor venæ jugularis internæ arise in place; they are not cut off from one of the larger adjacent muscles. This observation indicates that the constrictor muscle is not a recent acquisition but has been derived, by modification and change of function, from an earlier muscle which occupied a closely related position.

The probable character and function of this primitive muscle are suggested by a study of the branchial muscles of the lower amphibians. All *Perennibranchiata*, *Derotremata*, and *Cœcilia* are provided with a system of muscles which arise from the parotic region of the head and insert on the dorsal ends of the cartilaginous branchial arches (mm. levatores arcuum of Fisher, 54). According to Hoffmann, 73-78, there are four pairs of these muscles in *Siren*, three pairs in *Proteus*, *Necturus*, *Cryptobranchus*, and *Amphiuma*. Homologous muscles also occur among the *Salamandrida* (Coghill, 02).

In accordance with the close relation which exists between the glosopharyngeus and vagus nerves in the *Urodela*, the mm. levatores arcuum

may be innervated either wholly by the vagus, or partly by the vagus and partly by the glossopharyngeus. In *Necturus* all of the levator muscles are supplied with nerves which arise directly from the ganglion vagi (Fischer, 54).

These branchial muscles of the Urodela thus bear a striking resemblance to the constrictor muscle, both in innervation and attachment. Moreover, the constrictor muscle also plays the part of a branchial muscle in certain forms. It is, therefore, not improbable that the constrictor muscle of the Sauria is descended from one of the *mm. levatores arcuum*, or a muscle with a similar function, belonging to amphibian ancestors.

b. THE MUSCULUS PROTRUSOR OCULI.

I have been unable to find a description of this muscle in the literature dealing with the myology of the Sauria. Its general relations have been recently figured by Watkinson, 06 (Figs. 12, 13, 14, Plate XII), in a paper on the cranial nerves of *Varanus bivittatus*, but the muscle is mistaken for the *m. depressor palpebræ inferioris* of Weber, 77.⁷

The *m. protrusor oculi* of *Lacerta agilis* (*m. p. o.*, Figs. 4 and 5, Plate I) is closely related both to the sinus orbitalis and to the anterior part of the vena jugularis interna. The venter of the muscle is a triangular body which arises by a dorsal angle from the cranial wall anterior to the foramen trigemini. Its fibers spring from the posterior part of the tænia parietalis media, a cartilaginous rod (*T. p. m.*, Fig. 4, Plate I), which reinforces the lateral wall of the cranium above the proximal portion of

⁷The *m. depressor palpebræ inferioris* was first described by Fischer, 52, under the name *adductor maxillæ inferioris*. The muscle was considered a homologue of a palatine muscle of the snake and the *m. palpebralis* which Bojanus, 19-21, described in the turtle. The actual relations and function of the muscle were first recognized by Stannius, 56, p. 170, who says: "Das untere Augenlid wird durch eine flache, den Boden der Augenhöhle bildende Muskelausbreitung abwärts gezogen." In 77 the muscle was more fully described by Weber, who gave it the name *m. depressor palpebræ inferioris*. His description, which is based on *Lacerta*, is as follows:

"Der *m. depressor palpebræ inferioris* nimmt seinen Ursprung von dem unteren Rande des Septum interorbitale und zwar in der ganzen Breite desselben; oder genauer gesagt, von dem hinteren, unteren Winkel der Nasenwand, dem Palatinum, dem Praesphenoid, weiter von dem Pterygoid und dem unteren Rande der Fascie, welche sich zwischen der Augenhöhle und den Kaumuskeln ausdehnt. Lateralwärts schiebt sich der Muskel in der ganzen Breite der Augenhöhle zwischen dem bulbus und den Grund der Augenhöhle. Er setzt sich an dem unteren Rand des Tarsus, zum Theil auch an das Bindegewebe, welches diesem aufliegt, an."

the ramus ophthalmicus V. From their origin the fibers of the muscle descend between the temporalis muscle, on one hand, and the cranial wall, eye muscles, and the vena jugularis interna, on the other. The posterior part of the muscle lies medial to the columella (epipterygoid) and is composed of almost vertical fibers. The anterior fibers extend downward and forward to a point lateral to the optic chiasma, where the muscle is continuous with the caudal part of the m. depressor palpebræ inferioris. The fibers of the m. protrusor oculi terminate ventrally in a horizontal fascia which begins directly in front of the basisphenoid bone and extends forward under the vena jugularis interna and the sinus orbitalis. The anterior part of the fascia gives rise to the fibers of the m. depressor palpebræ inferioris and is attached in the median line to the septum interorbitale. Posteriorly the fasciæ of opposite sides unite to form a continuous sheet (*t.*, Fig. 5, Plate I), which is free in the middle line, excepting an occasional band (*t'*) which is attached to the cartilaginous basis cranii (compare also Fig. 4, Plate I). The posterior parts of the two protrusor muscles thus form practically a U-shaped digastric muscle with a middle fascia stretched below the two venæ jugulares internæ.

The relations and function of this muscle are so peculiar that I wish to call attention to some other forms in which the muscle is even more highly developed than in *Lacerta*. In *Monitor niloticus* (Fig. 2, Plate III; Fig. 1, Plate II) the protrusor muscle (*m. p. o.*) arises by a well-developed tendon, the posterior part of the muscle is everywhere attached in the middle line and the two muscles are entirely separate. The anterior fibers of the muscle extend far forward below the sinus orbitalis and terminate under the axis of the bulb, in a fascia which lies in the floor of the sinus.

In *Anguis fragilis* the m. protrusor oculi is also a strong muscle. It arises chiefly from the cranial wall dorsal to the proximal part of the ramus ophthalmicus V. The posterior part of the muscle extends downward to reach the ventral aspect of the vena jugularis interna, the anterior fibers stretch forward and spread out fan-like on the floor of the sinus orbitalis.

The posterior part of the m. protrusor oculi of *Phrynosoma* is shown in Fig. 1, Plate III. Here the muscle (*m. p. o.*) is relatively short and stout. Its dorsal portion arises from a bony process which extends forward from the lateral part of the basisphenoid bone, its posterior fibers terminate in a fascia which is inserted, partly on the trabecula cranii.

partly on a posterior process of the subiculum infundibuli of Gaupp, **oo**.

In addition to the forms previously mentioned the *m. protrusor oculi* has been observed in the following:

- Chamæleon vulgaris Cuvier.
- Agama colonorum Daudin.
- Moloch horridus Gray.
- Anolis caroliniensis Cuvier.
- Sceloporus undulatus Latreille.
- Platydactylus mauritanicus Linnaeus.
- Cnemidophorus sexlineatus Linnaeus.

The *m. protrusor oculi* is innervated by twigs from the ramus ad. *m. depressorem palpebræ inferioris* of Fischer, **52**, which springs from the motor portion of the ramus mandibularis V, passes downward and forward and enters the posterior ventral angle of the protrusor muscle (*r. d. p. i.*, Figs. 4 and 5, Plate I). Within the muscle the nerve gives rise to two or three *nervi protrusores oculi*, while the nerve trunk continues forward through the ventral part of the muscle to reach the *m. depressor palpebræ inferioris*.

The embryonic development of the *m. protrusor oculi* is contemporaneous with that of the other muscles of the orbital region. In a *Sceloporus* embryo with head 2100 μ long the first fibers are in process of formation and the muscle is functionally mature when the embryo is hatched. In the stages examined there is no evidence of a foreign derivation. The innervation, however, shows a close phylogenetic relation to the *m. depressor palpebræ inferioris*.

The functions of the *m. protrusor oculi* are sufficiently clear from its relations to the vena jugularis interna and the sinus orbitalis. By the contraction of its posterior portion the ventral fascia is elevated and the vena jugularis interna is compressed against the eye muscles, which form a sort of cushion between the vein and the subiculum infundibuli (*S. i.*, Fig. 5, Plate I). The anterior part of the muscle elevates, and gives tension to, the fascia which underlies the sinus orbitalis.

c. THE MUSCULUS PROTRUSOR OCULI ACCESSORIUS.

The *m. protrusor oculi accessorius* is a hitherto undescribed muscle which has been found in only a few forms. In *Monitor niloticus* (*m. p. o. a.*, Fig. 2, Plate III; Fig. 1, Plate II) the muscle is a broad sheet of striated fibers which fits loosely upon the posterior part of the bulbus, from which it is separated by the sinus orbitalis. The muscle arises chiefly

from the pila accessoria of Gaup, oo, a transverse cartilaginous rod (*P. a.*, Fig. 1, Plate II), which lies in the cranial wall dorsal to the ramus ophthalmicus V. Some of the lateral fibers of the muscle arise from the fascia which forms the posterior wall of the orbit. From its origin the m. protrusor oculi accessorius bends first ventrad, around the posterior part of the bulbus, then rostrad below the sinus orbitalis, where it lies under the m. depressor palpebræ inferioris and lateral to the anterior part of the m. protrusor oculi. Below the bulbus the fibers of the m. protrusor oculi and m. protrusor oculi accessorius terminate in a single fascia, which lies in the floor of the sinus orbitalis.

The m. protrusor oculi accessorius is innervated by fibers from the ramus ad m. depressorem palpebræ inferioris, a branch of which nerve passes from the m. protrusor oculi into the accessory muscle, where the two muscles lie side by side on the postero-ventral aspect of the bulbus.

The function of the m. protrusor oculi accessorius is similar to that of the anterior part of the m. protrusor oculi; it presses against the sinus orbitalis from behind and elevates the fascia which forms the floor of the sinus.

The m. protrusor oculi accessorius has been observed only in *Monitor niloticus* and *Platydactylus mauritanus*. In the latter the muscle is very strong, the dorsal attachment extending forward as far as the junction of the tænia marginalis with the solum suprasetale.

The innervation of the m. protrusor oculi accessorius indicates an origin either from the m. protrusor oculi or from the m. depressor palpebræ inferioris.

B. DISTENSION OF VEINS AND SINUSES AND ELEVATION OF BLOOD-PRESSURE IN THE HEAD OF SAURIA.

The immediate function of the m. constrictor venæ jugularis internæ is indicated clearly enough by its structure and relations; it has also been repeatedly demonstrated experimentally, both by direct stimulation and by stimulation of the nerve. As previously stated, the muscle includes both circular and longitudinal fibers. During the contraction of the muscle, therefore, two different movements may be seen: a constriction of the vein by the circular fibers and a longitudinal contraction of the vein by the longitudinal fibers. On account of the former the lumen of the vein is closed, the blood current is interrupted and the vein becomes pale and colorless. The longitudinal fibers close the mouths of the tributary veins and thicken the wall of the vena jugularis interna, thus facilitating the work of the circular fibers.

The closing of the jugular vein is also made easier by valves within the vein at this point. They are valves of the ordinary form, which are used under normal conditions to prevent a reversal of the blood current. When, however, the constrictor muscle contracts, they serve as a cushion which assists in blocking the lumen of the vein. Similar valves have been observed at this point in several genera, including *Lacerta*, *Agama*, *Monitor*, and *Moloch*.

The effect of the obstruction of the *vena jugularis interna* upon the circulation of blood in the head is naturally very marked. Under ordinary circumstances the vein probably receives nine-tenths of all the blood from the cranium, face, and jaws. When the vein is closed by the constrictor muscle, blood accumulates in the distal portion of the vein, its tributaries are distended and the venous blood-pressure rises. Within the cranium the large sinus-like veins are flooded, but on account of the rigid brain case the enlargement of these veins soon reaches its limit. In the extracranial sinuses, on the other hand, the distension increases as the blood-pressure rises and the total enlargement may be very great. The most conspicuous effects occur in the *sinus orbitalis*, whose distension is facilitated both by its direct connection with the *vena jugularis interna* and by the great extent of its drainage territory. From the *sinus orbitalis* the area of high blood-pressure spreads into the antorbital veins and into the *vena supratemporalis*, and thus completes the invasion of the head. These effects may be modified more or less by tonic contraction of certain orbital muscles, which thus tend to prevent the enlargement of the *sinus orbitalis* and to hasten the distension of the veins and other sinuses of the head.

The contraction of the *m. protrusor oculi* and *m. protrusor oculi accessorius* produces effects which are limited chiefly to the anterior part of the head. The first muscle, acting either alone or with the second, causes a rise of blood-pressure in the *sinus orbitalis* and elevates the *bulbus*. At the same time the *sinus orbitalis* and its tributaries are more or less distended. The precise effects may vary, however, under different conditions. A strong tonic contraction of the orbital muscles might prevent distension of the *sinus orbitalis*, while it would accelerate distension of the tributaries of the sinus. Relaxation of the orbital muscles is followed by gradual distension both of the *sinus orbitalis* and its tributaries. The contraction of the *protrusor* muscles is not favorable, however, under any conditions, for the greatest distension of the *sinus orbitalis*, on account of the pressure which they exert upon the wall of the sinus. The most striking effect of their contraction is to be observed when the

muscles act upon the already distended sinus orbitalis. This effect is considered later.

External evidence of the contraction of the muscles of the swell mechanism may be readily observed in the lizards. The distension of the superficial veins and sinuses produces external intumescence or swelling, which may become very pronounced in certain parts, especially in the region of the orbit. Evident movements also occur at the external nares, and in some forms these openings may be wholly closed by the distension of the sinus vestibuli nasi.

Some of the minor movements of this sort may be simple vaso-motor effects. More marked movements occur, however, which cannot be explained in this way. Their occurrence was first observed in *Anolis carolinensis*, the so-called chameleon of the southern United States. The movements began with a quiet swelling of the orbital region on both sides of the head, the enlargement increasing gradually and without apparent cause. Then came a spasmodic contraction of muscles, especially those of the lower jaw and pharynx, while at the same time the bulbus and eyelids were forcibly protruded, until the lids and loose skin about them were stretched to the utmost. At the maximum enlargement the diameter of the head across the orbits was increased by about one-third of the normal diameter. After this stage the parts were quickly restored to their usual condition and the swelling completely disappeared (compare Text Figs. 9, 10, and 11).

The conditions under which these movements occurred will be stated later; at the present time it is sufficient to say that they were repeatedly observed, so that the details could be carefully studied. At the time the movements were first noticed only the *m. constrictor venæ jugularis internæ* had been discovered. But the peculiar events of the second stage showed the participation of other muscles and finally led to the discovery of the *mm. protrusor oculi* and *protrusor oculi accessorius*. At the same time it was observed that the orbital swelling was accompanied by similar, though less pronounced, effects in other parts of the head.

These facts pointed to the existence of a complicated mechanism, consisting of muscles, nerves, and blood-vessels, all co-ordinated in a definite way to produce the observed results. On account of the character of these results the mechanism may be appropriately designated a *swell mechanism*. The nature and causes of the movements which it produces must now be studied in detail. For this purpose I shall use *Anolis carolinensis* as a type, but other forms will be frequently referred to. I begin with the protrusion of the eyes.

a. DISTENSION OF THE SINUS ORBITALIS AND PROTRUSION OF THE EYES.

As already stated in the preliminary description given above, the protrusion of the eye of *Anolis* includes two distinct stages:

In the first stage, or stage of distension, the sinus orbitalis gradually fills with blood and the bulbus is moderately protruded. No muscle movements are visible in the region of the eye or elsewhere, and the intumescence is apparently due wholly to blood-pressure. As a result of this pressure the eyelids are usually closed, chiefly by the elevation of the lower lid, while both lids become turgid with blood and lymph, the latter being forced, by the great blood-pressure, from the deeper sinuses and channels into the lymph-spaces of the lids.

The average duration of this stage is five seconds, but it may be either longer or shorter, the greatest length observed being about fifty seconds. With the average duration of this stage a maximum enlargement is attained which amounts to about three-fifths of the final maximum which occurs at the close of the second stage. With less than the average duration the amount of enlargement is less, while prolongation beyond the average results simply in the maintenance of a uniform state, which represents, therefore, the maximum effect possible under the prevailing conditions.

The second stage of orbital enlargement is a stage of high blood-pressure. It is characterized by a sudden protrusion of the eyes, energetic contraction of the muscles of mastication, and elevation of the floor of the mouth. The eyelids remain closed and the membrana nictitans is more or less protracted, presumably as a result of the great blood-pressure. The duration of this stage in *Anolis* is usually about one-half second.

1. ACTIVITY OF MUSCLES.

1. FIRST STAGE.—The flooding and distension of the sinus orbitalis during the first stage is due to a combination of several different causes. One of the most important of these is the *m. constrictor venæ jugularis internæ*. The contraction of this muscle during the first stage of orbital protrusion is shown by the following facts:

(1) The amount of protrusion and the rate of enlargement *both* indicate the obstruction of the *vena jugularis internæ*, the outlet of the sinus orbitalis. The flooding of this sinus is not simply a vaso-motor effect.

(2) The general intumescence which accompanies the protrusion of the orbital region locates the obstruction in the posterior cephalic region.

Outside of the orbital region more or less evident swelling occurs in

other parts of the head, from the nasal openings to the occiput. Especially significant, however, is the intumescence which occurs in the region of the external auditory depression, where the vena mandibularis and its tributaries lie close to the skin. The intumescence in this region is especially marked in those forms which are provided with a thin skin and small scales. In *Anolis*, for example, the swelling is accompanied by a considerable separation of the scales, while the thin integument between the scales is intensely reddened by the increase of blood in the subjacent veins. These phenomena are undoubtedly due to unusual turgescence of the vena mandibularis,—a vein which penetrates the constrictor muscle to enter the vena jugularis interna. These facts clearly point to the obstruction of the vena jugularis interna by the constrictor muscle.

Experimental evidence leads to the same conclusion. The artificial obstruction of both venæ jugulares internæ produces results which resemble in all respects, excepting perhaps in degree, the natural flooding of the sinus orbitalis and other cephalic veins during the first stage.

We may safely conclude, therefore, that the flooding and distension of the sinus orbitalis during the first stage is caused, in part at least, by the contraction of the m. constrictor venæ jugularis internæ. As a result of this contraction the jugular vein is blocked, the blood accumulates in the peripheral part of the vein and the escape of blood from the sinus orbitalis is prevented. The sinus itself is then distended, partly by blood which is poured into it by its numerous tributaries, partly by blood which comes directly from the capillaries. The amount of the distension is determined by the arterial blood-pressure, which keeps the blood flowing into the sinus until the venous pressure almost equals that of the local arteries themselves. At this point distension of the sinus ceases and the protrusion of the orbital region also reaches a maximum, in so far as it may be affected by the circulatory mechanism. Then follows the second stage.

2. SECOND STAGE.—During this stage the constrictor muscle maintains its tonus, as indicated by the persistence of the postorbital swelling. On account of the great number of muscles employed, the study of this stage presents a somewhat difficult problem. It is believed, however, that the following description contains all of the important factors, with the approximate function of each:

(1) *Musculus Protrusor Oculi* (*m. p. o.*, Figs. 4 and 5, Plate I, Figs. 1 and 2, Plate III, and Fig. 1, Plate II).—The immediate function of this muscle has been already stated. Its contraction during the second stage

cannot be directly observed, but it may be safely inferred from the character of the result.

During the first stage of orbital protrusion the sinus orbitalis is distended until the blood-pressure in the sinus reaches a maximum for the existing arterial pressure. During the second stage the outlet of the sinus is closed by the posterior part of the *m. protrusor oculi*. A considerable amount of blood is forced out of the enlarged anterior end of the vena jugularis interna and much of this blood enters the sinus orbitalis. At the same time the anterior part of the *m. protrusor oculi* presses against the wall of the sinus from below and behind. If the walls of the sinus were rigid outside of the territory affected by the muscle, contraction of the latter would produce a simple rise of blood-pressure. But since the lateral wall of the sinus is composed of elastic tissues a different result must follow; for whenever the pressure exerted by the *m. protrusor oculi* exceeds the blood-pressure existing in the sinus orbitalis, the floor and posterior wall of the sinus are pushed in, the lateral wall is pushed outward to a corresponding degree and the amount of orbital protrusion is increased.

More or less protrusion of the eyes under ordinary conditions may be readily produced by artificial pressure upon the roof of the mouth between the anterior ends of the pterygoid bones (compare Figs. 4 and 5, Plate I), where the vena jugularis interna lies near the mucous membrane.

Rathke, 66, assigns to the *m. depressor palpebræ inferioris* of the crocodile the function of elevating the bulbus under certain conditions. Such a movement would assist in the protrusion of the eye of the lizard during the second stage, but I have not been able to discover any evidence that the lid muscle contracts at this time. Moreover, my experiments on *Anolis* show, that after the sinus orbitalis is flooded stimulation of the *m. depressor palpebræ inferioris* causes simple depression of the lid, as under ordinary conditions.

(2) *Musculus Protrusor Oculi Accessorius* (*m. p. o. a.*, Fig. 2, Plate III, and Fig. 1, Plate II).—This muscle does not occur in *Anolis*, and no physiological observations have been made on any forms in which the muscle has been observed. In view of its position, however, there can be no doubt in regard to its function, which is the same as that of the anterior part of the *m. protrusor oculi*. In contraction it presses against the distended sinus orbitalis from below and behind, and thus by increasing the blood-pressure, augments the protrusion of the eye.

(3) *Musculus Temporalis* (*m. t.*, Fig. 1, Plate II).—The contrac-

tion of the *m. temporalis* during the second stage of orbital protrusion is easily seen in *Anolis* and the effect is not open to doubt. The muscle supports the fascia which forms the posterior wall of the sinus orbitalis. When the muscle is relaxed during the first stage of orbital protrusion, the blood-pressure in the sinus orbitalis pushes the muscle backward. In the second stage of protrusion the muscle contracts, its anterior portion presses against the flooded sinus orbitalis; the blood-pressure is raised and the protrusion of the eye is increased.

(4) *The Bucco-pharyngeal Muscles*.—No attempt has been made to analyze the movement caused by these muscles and its significance is not entirely clear. It is essentially a swallowing movement, and includes elevation of the floor of the mouth and contraction of the pharyngeal muscles. By elevating the floor of the mouth it is possible that pressure may be applied against the floor of the orbit, where the large sub-orbital foramen is closed only by soft tissues. Suitable pressure in this region by means of the tongue or hyoid apparatus would augment the protrusion of the eye, as may be easily demonstrated by artificial means. Efforts to observe such movements of the hyoid apparatus, however, were unsuccessful, for the attempt to hold the mouth open led in all cases to a suspension of the movements.

The contraction of the pharyngeal muscles probably assists in producing higher blood-pressure in the posterior part of the head. Such a result would probably follow from the pressure of the contracting muscles upon the flooded veins and sinuses.

In accordance with the above account, I conclude:

The protrusion of the eye of the lizard is caused by the distension of the sinus orbitalis and the elevation of blood-pressure in the same.

In the first stage of protrusion the observed effects are due, in a large measure, to the contraction of the *m. constrictor venæ jugularis internæ*. As a result of such contraction, blood accumulates in the sinus orbitalis and the blood-pressure rises until it reaches a maximum for the existing arterial pressure. This condition is ordinarily attained in *Anolis* in about five seconds, but the time required is subject to some variation.

In the second stage, or stage of high pressure, the *m. constrictor venæ jugularis internæ* maintains its tonus; the outlet of the sinus orbitalis is closed by the *m. protrusor oculi*, which at the same time pushes against the membranous wall of the sinus and raises the blood-pressure to a higher level. As a result of this the orbital protrusion is increased by stretching the elastic lateral wall of the sinus.

2. SECONDARY CAUSES AND CONDITIONS WHICH AFFECT THE DISTENSION OF THE SINUS ORBITALIS.

I have shown that the distension of the sinus orbitalis is largely an effect of the contraction of certain muscles (m. constrictor venæ jugularis internæ, m. protrusor oculi, m. protrusor oculi accessorius, and the buccopharyngeal muscles). There are also certain other factors which affect the distension of the sinus.

(1) *Acceleration of the Heart's Beat.*—In *Anolis* there is a marked increase in the number of cardiac pulsations during the distension of the sinus orbitalis. For example, in a specimen showing 112 beats per minute before protrusion began, the number of beats increased as distension increased, until the rate reached a maximum of from 135 to 149 pulsations per minute. The acceleration is not noticeable at the very beginning of distension but follows after a short interval. The maximum rate is attained approximately at the moment of maximum distension. If the stage of distension is prolonged, the rate of pulsation continues about the same but the beats become feeble, probably on account of exhaustion. After the stage of high pressure passes, the heart-beat becomes slower, but symptoms of exhaustion may be noticed for a short time.

The more rapid cardiac action undoubtedly raises the blood-pressure in the cephalic arteries and facilitates the distension of the sinuses and veins of the head. If other conditions remained uniform after obstruction of the vena jugularis interna, the amount of blood sent to the head would gradually decrease so long as the distension of the cephalic vessels continued. But when the heart begins to beat faster, the blood-pressure is diminished in the veins near the heart, the intake of blood from the posterior veins is increased and a larger amount is sent to the head at the expense of the posterior parts.

The acceleration of the heart-beat is presumably a reflex effect, due to stimulation of the cardio-accelerator center of the medulla. The origin of the stimulus has not been determined, but it may be accounted for, perhaps, either by the accumulation of impurities in the blood of the brain, or by the rise of blood-pressure within the cranial cavity. The first mode of acceleration occurs in the mammals (Foster, 94). The second method has apparently not been observed in this group, in which high blood-pressure in the cranial cavity produces an exactly opposite effect, the heart being slowed down by vagus inhibition. This last-mentioned reflex may account for the slowing of the heart of the lizard

after the second stage of intumescence. During the first stage the cardio-accelerator center evidently gets the upper hand.

(2) *Vaso-motor Adjustment of Arteries.*—In view of the increased activity of the heart during the distension of the veins and sinuses, it is probably safe to assume the vaso-dilation of the carotids and their cephalic branches. Indeed, a mechanical distension of these arteries would naturally follow from higher blood-pressure alone, unless it were prevented by constriction of the vessels.

I have not been able to discover a direct opening of arteries into the sinus orbitalis. The arteries which feed the sinus break up into capillaries, which run a short distance through the tissues and then widen out gradually to open into the sinus. Even under such conditions, however, the dilation of the arteries leading to the sinus would hasten a process which, at best, involves more or less disturbance of the normal functions of the eye.

The accumulation of an extraordinary amount of blood in the head probably requires, also, constriction of the posterior arteries of the body. During the distension of the cephalic veins and sinuses of *Anolis*, the quantity of blood in the head is probably doubled. The extra amount, which is equal to one-sixth of the total amount in the body, must be withdrawn from the posterior parts. This would seem to require extensive vaso-motor changes, including both dilation of the arteries leading to the head and constriction of the posterior arteries.

(3) *Striated Muscles of the Orbit.*—Under ordinary conditions a slight dilation of the sinus orbitalis follows the relaxation of certain striated muscles of the orbit, especially the m. retractor oculi and the m. depressor palpebræ inferioris (m. adductor maxillæ inferioris, Fischer, 52). This fact is easily demonstrated by the use of curare. About one drop of a 1 per cent solution injected hypodermically in the dorsal trunk region is followed by a perceptible protrusion of the eye in about five minutes. In twenty minutes the control of the voluntary muscles is lost, the lower eyelid rises, the bulbus protrudes, and the orbital enlargement is quite marked.

This experiment shows:

(a) That ordinary blood-pressure in the sinus orbitalis is sufficient to produce a certain amount of distension of the sinus. Such pressure, indeed, is the most important factor in the closing of the eye, which is due chiefly to the elevation of the lower eyelid. Under ordinary conditions the m. depressor palpebræ inferioris maintains a certain tonus sufficient to force the blood out of the palpebral part of the sinus orbit-

alis and to keep the lower lid depressed. When the muscle relaxes, the blood enters the palpebral space and elevates the lid. This fact was observed long ago by Weber, 77.⁹

(b) The distension of the sinus orbitalis under ordinary blood-pressure is prevented by the striated muscles of the orbit. By raising the tonus of these muscles, therefore, the distension of the sinus may be more or less prevented even under extraordinary blood-pressure. This explains the difficulty sometimes experienced in attempts to flood the sinus orbitalis by artificial compression of the venæ jugulares internæ.

Whether all of the bulbus muscles are concerned in producing these results must be left undecided. Retraction of the bulbus is generally supposed to be a special function of the m. retractor oculi, but according to Weber, 77, this muscle is assisted by the m. bursalis. It is evident, moreover, that the recti and obliqui might play a similar rôle.

(4) *Musculus Compressor Sinus Orbitalis*.—This muscle, which was first described by Leydig, 72, is a sheet of smooth fibers which arises in the median part of the orbit and extends in a meridional direction around the bulbus and into the eyelids (*m. c. s. o.*, Fig. 2, Plate III, Fig. 1, Plate II). The muscle lies everywhere outside of the sinus orbitalis, for which it forms an almost complete covering. Below the bulbus the muscle lies on the dorsal side of the m. depressor palpebræ inferioris, but it is weakly developed in the region of this muscle, which seems to some extent to take the place of the smooth muscle in its relation to the sinus orbitalis. In the neighborhood of the canthi, where the smooth muscle is very strong, it includes both meridional fibers and a second layer which is composed of vertical fibers (Fig. 2, Plate III). The smooth muscle also reaches into the membrana nictitans and envelopes the sinus membranæ nictitantis.

At the proximal border of the lower eyelid the smooth muscle divides into two parts, one of which passes between the tarsus and the conjunctiva, while the other runs through the trabeculæ of a great lymph-sinus which lies next to the cutis plate of the lid. A similar lymph-sinus occurs in the upper eyelid, where also the smooth muscle is well developed.

⁹ According to Weber, the closing of the eye may be more or less accelerated by two other factors: (1) Elasticity of the tissues of the lower eyelid. When the m. depressor palpebræ inferioris relaxes, the folded parts of the lid, including both conjunctival and cutis plates, tend to unfold and elevate the lid. (2) Retraction of the bulbus causes a rise of blood-pressure in the sinus orbitalis and thus hastens the elevation of the lid. This would evidently tend to depress the upper eyelid also.

As observed by Leydig, 72, p. 81, the smooth muscle of the orbit has nothing to do with the ordinary winking movements, which are confined to the lower eyelid. Leydig assigns to the muscle the function of expelling the glandular secretions of the eye. Weber, 77, concludes, from the distribution of the muscle fibers in the eyelids, that the muscle is used to drive out the lymph from the sinuses of the lids. The latter view is undoubtedly correct, in so far as it concerns the palpebral portions of the muscle. This function, however, does not explain the existence of the deeper part of the muscle, to which another office must be assigned, namely, the compression and reduction of the flooded sinus orbitalis. Such a function is clearly indicated by the direction of the fibers and by their relation to the sinus orbitalis.

It is not improbable, especially in view of the character of the muscle, that it maintains, under ordinary circumstances, a certain tonus and thus assists in preserving normal conditions in the sinus orbitalis. The relaxation of the muscle would, therefore, facilitate the flooding of the sinus orbitalis, after the contraction of the *m. constrictor venæ jugularis internæ*.

In addition to the functions just described the *m. compressor sinus orbitalis* produces certain peculiar movements of the eyelids. In a specimen of *Sceloporus undulatus*, for example, a peculiar contortion of the upper lid was noted, the movement beginning at the anterior canthus and advancing wave-like toward the posterior canthus. The progress of the movement was slow and resembled the movements which occur at the external nares. Since the upper eyelid is provided only with smooth muscle fibers from the *m. compressor sinus orbitalis*, that muscle must be the cause of the movement. Similar movements which were observed in the lower eyelid are undoubtedly to be attributed to the same muscle.

b. REDUCTION OF THE SINUS ORBITALIS.

If the relaxation of the orbital muscles facilitates the flooding of the sinus orbitalis, the contraction of these muscles, after the sinus has been flooded, must accelerate the escape of blood and assist in reducing the sinus to its ordinary condition. The contraction of the bulbus muscles forces the blood especially from the deeper parts of the orbit, while the smooth orbital muscle, *m. compressor sinus orbitalis*, exerts a pressure throughout the entire orbit, expelling the blood both from the sinus orbitalis and from the sinus *membranæ nictitantis*, and reducing the swollen eyelids by compression of the great lymph-sinuses.

In these changes the *m. depressor palpebræ inferioris* also plays a part

by elevating the floor of the sinus orbitalis, depressing the lower eyelid and retracting the adjoining skin. These functions probably explain the relatively strong development of this muscle, especially in the neighborhood of the posterior canthus.

Slowing down of the heart-beat and vaso-motor adjustment of the arteries probably contribute to the reduction of the sinus orbitalis by limiting the supply of the blood which enters the sinus.

The elasticity of the tissues is probably a factor of some importance in the reduction of the sinus orbitalis but such action is naturally limited to the early stages of the process.

c. DISTENSION OF THE SINUS VESTIBULI NASI.

When the eye of the lizard is protruded, a sympathetic swelling may sometimes be observed in the region of the sinus vestibuli nasi. The cause of this swelling is clearly indicated in certain forms (*Sceloporus*) by the occurrence of distinct phases corresponding to those observed in the sinus orbitalis. During the first phase the swelling is slow and gradual, but the second phase is marked by the arrival of a pulse-like wave which produces a conspicuous narrowing of the nasal opening.

These observations show that the flooding of the sinus vestibuli nasi may be effected by the same mechanism that is used for the distension of the sinus orbitalis and other sinuses of the head. This sympathy does not always manifest itself, however. On the contrary, the intumescence at the external nasal opening may occur without a general flooding of other sinuses of the head, or vice versa, a general flooding of the cephalic sinuses and veins may occur without distension of the sinus vestibuli nasi. These facts may be readily understood by reference to the description of the sinus vestibuli nasi on page 12. It is there shown that the possibility of local control of the sinus is vested in the smooth muscle fibers of the trabeculæ and arteries. The fact that the sinus maintains its ordinary contracted state after obstruction of the vena jugularis interna may be explained by a higher tonus of the muscle fibers of the trabeculæ and greater constriction of the arteries. The distension of the sinus under ordinary conditions may be accounted for by relaxation of the smooth muscles of trabeculæ and arteries.

These arrangements for local control probably account for the ordinary changes which occur in the sinus vestibuli nasi. It is possible, however, that the sinus may be distended by contraction either of the *m. constrictor venæ jugularis internæ* or the *m. protrusor oculi*, and if

the orbital muscles maintain a proper tonus, such distension may occur without enlargement of the sinus orbitalis.

The provision for local control of the sinus vestibuli nasi is evidently of considerable importance. It affords, on the one hand, the possibility that the external nasal openings may be constricted or closed without interfering with other functions of the head. On the other hand it permits a general distension of the cephalic veins and sinuses without disturbing the olfactory and respiratory organs.

More or less pronounced movements, due to the distension of the sinus vestibuli nasi, are of common occurrence among the lizards. In *Lacerta* the spongy tissue is unequally developed around the margin of the ex-

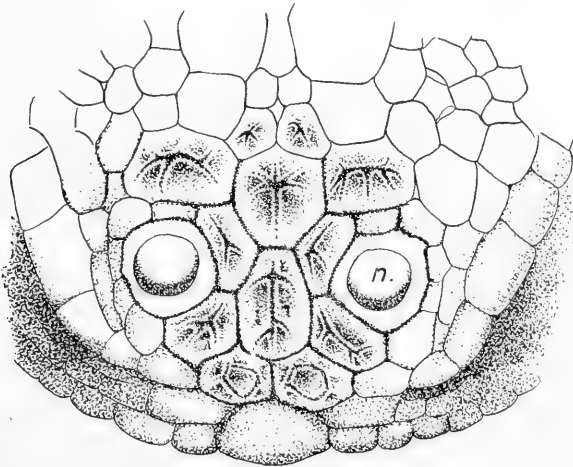


FIG. 8. Rostrum and nasal openings of *Phrynosoma cornutum*.

The openings are almost closed by spongy tissue, which forms a prominent cushion, *n*, at the posterior margin of each opening.

ternal naris and the visible movements are limited chiefly to the posterior and ventral parts of the opening. Complete closing of the external opening does not occur, but conditions are favorable for the closing of the deeper part of the nasal vestibule, where the spongy tissue is better developed. In *Phrynosoma* the spongy tissue completely surrounds the external naris, but it is much thicker about the posterior margin of the opening (*n*., Text Fig. 8). When the sinus vestibuli nasi is distended this part of the spongy tissue (*st*., Fig. 5, Plate II) swells up, cushion-like, and closes the opening. This function of the spongy

tissue is very important in this form which is known for its habit of burying itself in loose sand or earth (Boettger, 79).

In *Chamaeleon vulgaris* the spongy tissue is somewhat equally distributed around the nasal opening and the following movements have been observed: (*a*) Elevation and depression of the margin of the nasal opening; (*b*) changes in the form of the opening, due to unequal expansion of different parts of the spongy tissue; (*c*) waves of expansion from within outward. These sometimes surrounded the entire opening or they were limited to one side of the opening.

The resemblance of this spongy tissue of the nasal vestibule of the lizard to the erectile tissue of the reproductive organs has been remarked by Leydig, 72, p. 92, and Born, 79, both of whom, however, failed to report the presence of smooth muscle fibers. A preliminary note calling attention to these fibers was published by the writer of this paper in 97. The smooth muscle fibers have also been observed by Osawa, 98, pp. 304, 348, in *Hatteria*.

It is worthy of note in this connection that a spongy tissue similar to that of the nasal vestibule of the lizard occurs in the region of the inferior turbinate bone of mammals. Such tissue has been observed by Isch Wall, 87, in the mole, armadillo, pig, rat, and cat, while its presence in man has long been known to anatomists.

d. DISTENSION OF THE SINUS PALATINUS AND SMALLER SINUSES OF THE HEAD.

The phenomena above described are the most conspicuous effects which follow the obstruction of the vena jugularis interna and the elevation of blood-pressure in the head. Somewhat similar effects must, however, be produced in the sinus palatinus and in the numerous smaller sinuses and veins of the head. Evidence of the gradual distension of the superficial veins, such as the vena mandibularis, may be readily seen during the first stage. The high blood-pressure of the second stage also shows its characteristic effects. In an anterior direction a pulse-like wave runs through the vena maxillaris and causes distension of the sinus vestibuli nasi. Other vessels of this region are undoubtedly affected in the same way. The compression of the anterior part of the vena jugularis interna by the *m. protrusor oculi* must produce a similar, though less pronounced wave in the postorbital region, but owing to unfavorable conditions, especially because of the muscular movements which occur at this time, I have been unable to discover a pulse in the veins of that region. This may be explained in part, also, by the action of the bucco-pharyngeal

muscles, which probably compress the veins of the posterior part of the head, and thus, by raising the blood-pressure, tend to neutralize the effects of the contraction of the *m. protractor oculi*.

The significance of the large palatine sinuses is not entirely clear. On account of their numerous anastomoses they serve to equalize the blood-pressure in different parts of the head. Under ordinary conditions they may be used as reservoirs for the storage of blood, which, after obstruction of the *vena jugularis interna*, is forced out to assist in the distension of the more dorsal vessels. On the other hand, however, it may be necessary to consider the palatine sinuses as mere incidents of a process which has for its object the protrusion of the eye and distension of other veins and sinuses of the head.

The function of the *sinus dentalis* is also quite obscure. After obstruction of the *vena jugularis interna* it possibly serves as a reservoir for the overflow of blood from the mandibular veins.

e. LYMPH MOVEMENTS CAUSED BY VARIATION IN BLOOD-PRESSURE IN THE CEPHALIC VEINS AND SINUSES.

The occurrence of large lymph-sinuses in the eyelids of *Lacerta* has been mentioned in the preceding account. Lymph-sinuses are, however, not confined to the eyelids. The sinuses of the lids are simply part of an extensive system which invades the orbit and occupies the soft tissues in various parts of the head. One of the largest of the orbital sinuses, *sinus supraocularis*, communicates freely with the sinus of the upper lid. It lies above the *bulbus*, between the smooth orbital muscle and the supra-orbital bones. This sinus extends forward under the skin as a system of anastomosing spaces which gradually disappears in front of the eye. Posteriorly the supraocular sinus accompanies the *vena supratemporalis* a short distance from the orbit.

The lymph-sinus of the lower eyelid is broadly connected with an inferior orbital sinus, which lies between the smooth orbital muscle and the bones which underlie the lateral half of the orbit. The sinus of the lower lid also extends forward on the dorsal aspect of the lachrymal duct, and this extension is accompanied by fibers of the smooth orbital muscle, which lie between the sinus and the skin. In a posterior direction the sinus of the lower lid and the inferior orbital sinus both open into a system of lymph-spaces which extends through the subcutaneous tissue of the side of the head. These spaces are especially numerous around the external auditory depression, from which they continue caudad into

the neck. Similar subcutaneous spaces exist in the supraoccipital region.

The high blood-pressure which distends the capillaries, veins, and sinuses of the head, produces at the same time extensive movements of the lymph. Such a movement is well illustrated in the case of the lymph-sinuses of the orbit and eyelids. Owing to the distension of the sinus orbitalis the lymph is forced out of the deeper sinuses of the orbit; the large supraorbital and inferior orbital sinuses are emptied and a large part of the escaping fluid enters the sinuses of the eyelids, thus producing the characteristic swelling of the lids which accompanies the distension of the sinus orbitalis. Similar movements on a smaller scale probably occur in all of the soft parts of the head, the distension of the deeper blood-vessels causing a flow of lymph toward the subcutaneous sinuses, while at the same time the movement of the lymph toward the trunk region is accelerated. Both of these movements are probably augmented in another way. Under high blood-pressure the rate of transudation from the blood-vessels is probably accelerated and the total amount of the lymph in the tissues is increased.

After the reduction of the distended veins and sinuses and the restoration of normal blood-pressure, the lymph-pressure also falls and the lymph finds its way back to the deeper sinuses and spaces. The lymph-sinuses of the eyelids are emptied by their smooth musculature, the lymph returning, for the most part, into the supraorbital and inferior orbital sinuses, which, as already stated, lie external to the *m. compressor sinus orbitalis*. The orbital lymph-sinuses thus provide in a most convenient way for the prompt flooding of the sinuses of the eyelids, the distension of the latter following automatically, as it were, whenever the sinus orbitalis is flooded.

f. SUMMARY OF EVENTS WHICH OCCUR DURING THE OPERATION OF THE SWELL MECHANISM.

The chief events which occur during the operation of the swell mechanism in *Anolis* may now be briefly summarized as follows:

I. First stage of intumescence.

1. Contraction of the *m. constrictor venæ jugularis internæ*; relaxation of the orbital muscles. The constrictor muscle obstructs the chief efferent blood-vessel of the head and causes distension of veins, sinuses, and capillaries. As a result of this the lymph flows toward the superficial spaces, while at the same time the rate of transudation is probably increased.

2. The rise of blood-pressure is followed by acceleration of the beat

of the heart, which thus augments all of the processes named above. Vaso-motor adjustments probably produce like effects. The general result is more or less evident swelling of the soft parts of the head, which reaches its maximum in the orbital region where the enlargement is facilitated by relaxation of the orbital muscles. At the end of this stage, under ordinary conditions, a maximum enlargement is reached, which indicates that the distension of veins has attained its limit under the existing arterial pressure.

II. Second stage of intumescence.

3. Contraction of *m. protrusor oculi*, *m. temporalis*, and the bucco-pharyngeal muscles. During this stage the *m. constrictor venæ jugularis internæ* maintains its tonus and the venous blood-pressure is raised to a higher level. In the anterior part of the head this is due especially to the action of the *m. protrusor oculi* and *m. temporalis*, which press upon the flooded sinus orbitalis and thus send a wave of high pressure through the anterior veins. A similar elevation of blood-pressure occurs also in the posterior part of the head, where it is probably caused by the combined action of all of the muscles mentioned above. External evidence of the higher blood-pressure is most plainly visible in the orbital region, where the protrusion is greatly increased. A pulse-like movement may also occur at the external naris, where it is caused by the distension of the sinus vestibuli nasi.

III. Stage of reduction.

4. Relaxation of the *m. constrictor venæ jugularis internæ*, *m. protrusor oculi* and bucco-pharyngeal muscles; contraction of the muscles of the orbit; reduction of the flooded veins and sinuses.

5. The decline of blood-pressure is followed by a slowing down of the heart-beat, probably also by vaso-motor changes which aid in restoring the circulation to its normal condition.

The foregoing events form a normal cycle of intumescence. This is the usual process, which includes two well-marked stages. An incomplete cycle sometimes occurs with omission of the second stage. Occasionally, also, the movements of the second stage have been observed, without any evidence of previous contraction of the *m. constrictor venæ jugularis internæ*, the result being a moderate protrusion of the eye.

g. GENERAL REMARKS ON THE DISTENSION OF THE CEPHALIC VEINS AND SINUSES OF THE SAURIA.

1. During the first stage of intumescence, following the contraction of the *m. constrictor venæ jugularis internæ*, the blood-pressure in the cephalic veins and sinuses is modified in a variety of ways.

(a) At a given time the venous pressure varies in different parts of the head. If all of the efferent blood-vessels of the head were obstructed, approximate uniformity of blood-pressure would soon be established. But after the obstruction of the vena jugularis interna the smaller blood-vessels of the posterior head region still continue to carry blood, the quantity of which is greatly increased on account of the obstruction of the larger vein. For this reason the venous pressure in the posterior part of the head is somewhat lower than elsewhere, and complete equalization of blood-pressure is impossible. For the same reason, also, the venous pressure can never quite equal the arterial pressure, although the difference decreases toward the anterior part of the head.

(b) After the obstruction of the vena jugularis interna the blood-pressure rises in the veins until the acting efferent vessels of the head carry the same amount of blood as the afferent vessels. At this point a new mean pressure is established and the distension of blood-vessels reaches its limit. This condition of equilibrium is attained, under ordinary conditions, at the close of the first stage. It is due to an increase in the amount of blood transmitted by the acting veins, especially the vena jugularis externa, vena trachealis, perhaps, also, the vena spinalis.

(c) Other things being equal, the mean pressure in the cephalic veins and sinuses is finally determined by the rate and force of the heart-beat, by the amount of dilation of the carotids and their branches, by the amount of constriction of the posterior arteries, and lastly, by the general arterial pressure, which is a resultant of all the preceding and other conditions, which need not be mentioned here.

2. The second stage of blood-pressure, due to the contraction of the *m. protractor oculi* and associated muscles, is of short duration. The most marked effects occur in the sinus orbitalis, but a wave of high pressure also sweeps through the connected veins and sinuses. The occurrence of this wave indicates a blood-pressure which at least exceeds that already existing in the veins,—higher perhaps than that of the local arteries themselves. Since, however, the outlet of the sinus orbitalis is closed during this stage, the blood-pressure reaches a higher level in the sinus and its tributaries than in the other veins of the head. This seems to explain the relatively greater development of blood sinuses in the anterior part of the head, a fact already noted in the first part of this paper.

3. In this connection a word may be said in regard to a modification of the swell mechanism which occurs in *Chamaeleon vulgaris* and *Platydactylus mauritanicus*. In both of these forms the *m. protractor oculi*

is present and *Platydactylus* has also the *m. protrusor oculi accessorius*. In both species the venous sinuses are well developed in the orbital region and in the anterior part of the head. On the other hand, the *m. constrictor venæ jugularis internæ* is entirely wanting and the postorbital veins are little, or not at all, enlarged. The operation of this modified mechanism must be more or less peculiar, but no observations have been made on the mechanism in action.

C. ON THE SIGNIFICANCE OF THE SWELL MECHANISM OF THE SAURIA.

a. A MOULTING MECHANISM.

The first theory entertained by the writer in regard to the final significance of the swell mechanism of the head of the Sauria was suggested by the well-known habit of many lizards to inflate the body for protective purposes or for other reasons. Carus, 34, calls attention to the inflation of the mouth or special laryngeal sacks as a means of sexual attraction or for frightening enemies. Leydig, 72, says that *Lacerta* enlarges the body by strong inflation of the lungs, and a similar habit occurs also in *Phrynosoma*, in which it serves to erect the numerous dorsal spines. According to Dekay, 42, *Sceloporus* elevates its spines when irritated, so as to present a formidable appearance. Still more remarkable adaptations, presumably for the same purpose, are the "frills" of *Chlamydosaurus kingii*, the gular sacks of *Metopocerus cornutus*, and many other *Iguanidæ*, the dilatable occipital sack of *Basiliscus*, etc.

In all of these cases it is reasonable to suppose that the effect might be augmented by enlargement of the head, and especially by the protrusion of the eyes. It has been suggested by Hay, 92, that *Phrynosoma* ejects blood from its orbital sinus as a means of frightening its enemies, and it is not improbable that in this case, the mechanism for elevating the blood-pressure is used as a *fright mechanism*. Such a function, however, is not sufficient to explain the wide distribution of the mechanism. On the contrary, it is probable that the flooding of the cephalic sinuses for frightening enemies is at best only a secondary use which has been acquired by relatively few forms.

There is still less evidence that the swell mechanism is used for sexual purposes. The mechanism is equally developed in both sexes and there is no reason to believe that it is employed as a means of sexual attraction.

A very important function of the swell mechanism of the Sauria has been discovered by a study of the moulting habits of these reptiles. Observations pointing to such an explanation were first made by the author, 98,

on *Anolis carolinensis*. A number of specimens of this species had been obtained for study and some of them were moulting. One of the animals had already removed the old skin from the trunk and occiput, but the anterior part of the head was still covered. My attention was first attracted to this specimen by its attempt to remove the old stratum corneum by scratching the side of the head with the hind foot. A fragment of loose epidermis was hanging from the lower eyelid, which twitched at intervals as if to remove an irritating object. The scratching was repeated several times, but without entirely removing the piece of epidermis. Then the animal became quiet, the eyelids closed and the entire



FIG. 9. *Sceloporus undulatus* in ordinary resting condition, for comparison with Figs. 10 and 11. $\times 3/2$.

orbital region began to swell, the enlargement proceeding with two distinct stages, as already described.

This entire series of movements, protrusion of both eyes, and scratching of the side of the head, was repeated at intervals until the irritating epidermis was finally removed. The movements then ceased.

On another occasion the same movements were observed in a moulting specimen of *Sceloporus undulatus*, all the details being reproduced which had been noted in *Anolis*, except the scratching. A substitute for this was found by rubbing the side of the head against the containing box. The swell movements were afterward duplicated by other specimens, both of *Sceloporus* and *Anolis*. In all cases they were directed toward the removal of the exuviae and ceased when the object was accomplished.

In the study of these moulting movements I experienced considerable difficulty at first on account of the failure of the supply of suitable material. This obstacle was finally overcome, however, by the application of court plaster, or similar material, to the head of the lizard. Such *artificial exuvia* formed a perfect substitute for the old stratum corneum and induced movements which were identical in every way with those which occurred under natural conditions. Protrusion of the eyes, with both stages well developed, scratching of the head or rubbing against a convenient object were all observed, the reaction following as promptly in specimens which had recently moulted as in those which were in the



FIG. 10. *Sceloporus undulatus*, showing orbital protrusion of second stage.
 $\times 3/2$.
 Court plaster on left upper eyelid.

midst of the moulting process. Text Figs. 10 and 11 are from specimens of *Sceloporus undulatus* which were treated in this way. They show fairly well the amount of enlargement at the moment of maximum orbital protrusion.

By the use of the court plaster method I have been able to observe the moulting movements in a considerable number of species and individuals, and in the same individual under different conditions. In all cases observed the mechanism was not set in motion until the plaster began to dry. In some species, such as *Anolis carolinensis* and *Sceloporus undulatus*, the response was usually very prompt, but shy indi-

viduals sometimes endeavored to execute the movements with the eyes open and alert. In other species satisfactory results were obtained only after prolonged observation.

In the same individual the energy and promptness of the response varied according to the point of application of the plaster; the movements occurring more quickly when the sense organs were affected, although the same reaction followed the application of plaster to other parts of the head.



FIG. 11. *Sceloporus undulatus*. Another specimen showing protrusion of second stage. $\times 3/2$.

The intervals between successive movements were also quite variable. In a given individual the movements were usually repeated with greater frequency soon after the plaster became dry. If the plaster was not removed as a result of these movements the intervals became longer. For example, in a specimen of *Anolis* nineteen cycles of intumescence were observed within one hour, but ten of these occurred during the first ten minutes of the period. In this case the movements began five minutes after the application of the court plaster.

Additional peculiarities of behavior in different species are given in the following list, which includes all species treated by the court plaster

method, excepting those already mentioned (*Sceloporus undulatus* and *Anolis carolinensis*). In the study of these forms a fairly uniform mode of treatment was employed, the court plaster being applied behind the posterior canthus, so as to leave the eyelids free.

1. *PHRYNOSOMA CORNUTUM*.—In this species the flooding of the veins and sinuses was somewhat slow, in accordance with the great deliberation which usually characterizes the movements of this form. Satisfactory results were easily obtained, however, both stages being shown by marked protrusion of the eyes. Special interest was attached to this experiment, because *Phrynosoma* is noted for the ejection of blood from the "eye." No ejection accompanied the moulting movements, however, although several specimens were subjected to the treatment and all responded with large protrusion of the orbital region.

2. *STELLIO VULGARIS* responded to the artificial stimulus at first with a mere twitching of the eyelids. Afterwards a quiet flooding of the sinus orbitalis was observed but reduction occurred without the production of a second stage. A few minutes later, however, the sinuses were flooded again, with two typical stages, both of which were well marked. Unusual prolongation of the intumescence was observed, the sinus orbitalis remaining distended for several seconds, as in the first stage. Occasionally, also, the second stage was repeated two or three times in succession, or with short intervals between, during which the sinus was kept distended, as in the first stage. After these movements the side of the face was usually scratched energetically with the hind foot.

3. One of the obstinate cases which finally yielded satisfactory results was that of *LACERTA MURALIS*. Observations were begun on this species in the summer of 1903, when several specimens in good condition were treated in the usual way. The response usually began with a rubbing of the head against a convenient object, while the eyes were moderately protruded. Another characteristic movement was the moistening of the lips with the tongue, which was pushed out between the lips and repeatedly drawn from the rostrum toward the angle of the mouth. Quiet flooding of the cephalic sinuses was observed and occasionally quick movements of the masticatory and bucco-pharyngeal muscles also occurred, but these movements were weak and not in their proper relation, so that the flooding of the blood-vessels was very incomplete.

The failure of these first experiments was accounted for by suppression of the regular movements, due to the shyness of the specimens treated. I accordingly obtained other specimens the following summer, and these, after some preliminary irregular movements similar to those

described above, gave a very satisfactory exhibition of the regular moulting movements, with complete protrusion of the eyes and both stages well developed.

4. *CNEMIDOPHORUS SEXLINEATUS* behaved in much the same way as *Lacerta muralis*, but after some delay typical results were also obtained in this species.

The difficulties encountered in this case and in *Lacerta muralis* may, perhaps, be explained by the unnatural conditions. It is not improbable that in many cases, at least, the actual removal of the exuvia is effected in retirement, and that under other conditions the moulting movements are performed only occasionally, or as a matter of necessity. The instinct to seek seclusion at the approach of the moulting time occurs in snakes, and it would apparently tend to develop also among the lizards, since the flooding of the sinus orbitalis necessitates at least partial closing of the eyes and thus exposes the moulting animal to more or less danger from enemies.

5. *UTA STANSBURIANA* and *SCELOPORUS SPINOSUS* appeared somewhat shy at first, but after a short interval the moulting movements were executed in a typical way.

6. *PLATYDACTYLUS MAURITANICUS* and *EUMECES FASCIATUS* were extremely shy and failed to respond to the court plaster treatment.

The preceding observations and the earlier studies on *Anolis carolinensis* and *Sceloporus undulatus* show that the habit of flooding the cephalic veins and sinuses for moulting purposes is well established among the Sauria. The typical process is the normal cycle of intumescence already described, which includes two distinct stages. The first of these is characterized by gradual flooding and distension of the veins and sinuses, with a corresponding intumescence of the soft parts of the head. The second stage is marked by a sudden increase of blood-pressure in the distended vessels, the protrusion of the eyes is increased and a wave of high blood-pressure runs through all venous vessels of the head.

This complicated process occurs naturally in response to the stimulus of the exuvia, or it may be induced in an experimental way by the application of court plaster or other suitable material to the head. The response, however, has the same physiological significance whether it follows the natural or the artificial stimulus, and is in all cases distinctly a moulting process.

b. GENERAL CHARACTERISTICS OF THE MOULTING PROCESS.

A discussion of the work of the moulting mechanism naturally requires, first of all, a review of certain anatomical peculiarities of the

skin, descriptions of which are to be found in the works of Leydig, 72, 73, Cartier, 74, Todaro, 79, Batelli, 79, and Blanchard, 80.

One of the most striking characteristics of the skin of the Sauria and other reptiles is the great thickness of the different layers. This is especially true of the outer stratum of the epidermis, the stratum corneum. This stratum is a protective covering, apparently a special adaptation to life in the air. In the vertebrate series it begins as a weak layer in the amphibians, where it is associated with glands which moisten the skin. In the reptiles the cutaneous glands are wanting and the protection of the body is delegated wholly to the stratum corneum, which is very thick and compact. In birds and mammals the glands of the skin reappear, the skin is protected by special epidermal outgrowths, and the stratum corneum is more or less reduced.

In all of these groups the outer layers of the epidermis are subject to a process of regeneration. In the mammals, birds, and some reptiles the stratum corneum wears away gradually and is renewed in the same way from the stratum Malpighii. In the Sauria, Ophidia, and Amphibia, on the other hand, the stratum corneum remains practically intact until it is removed, either as a whole or in fragments, by a process of moulting which extends over the whole body. This process is associated with a peculiar structure of the epidermis which must now be described.

Following the classification of Todaro and Blanchard we may distinguish in the epidermis of the Sauria the following subdivisions:

I. Stratum corneum.

(1) Pellicula epidermica.

(a) Stratum sculptum.

(b) Stratum internum.

(2) Stratum compactum.

(3) Stratum relaxatum.

II. Stratum Malpighii.

Of these different strata the pellicula epidermica is especially concerned in exuviation. As described by Blanchard and Leydig, it includes two divisions; a deeper one composed of a single layer of cells, and an outer homogeneous stratum sculptum, which is apparently a secretion of the underlying cells. This superficial stratum is ornamented with a peculiar sculpture which varies considerably in different families. A very simple condition occurs in *Platydactylus*, in which the surface of the pellicula is covered with closely crowded tubercles which measure about 1μ across the base. They are arranged for the most part irregu-

larly, but occasionally they occur in more or less sinuous rows. The stratum sculptum is more or less transparent and in places the outlines of the underlying cells can be clearly seen.

A different sculpture occurs in *Lacerta*, in which Leydig, 73, described a system of wave-like ridges on the surface of the pellicula. In *Lacerta agilis* and *muralis* these ridges are unsymmetrical, with a long slope of about 10° on one side while the other side is vertical or overhanging. The ridges are generally parallel in direction but adjacent ridges sooner or later anastomose, thus dividing the surface of the epidermis into more or less linear areas which vary from 10μ to 100μ or more in length and from $3\frac{1}{2} \mu$ to 5μ in width. The cells underlying the stratum sculptum can occasionally be seen also, the edges fitting together without overlapping. They average about 22μ in diameter and are generally independent of the superficial sculpture.

In sections of the skin the crests of the ridges show a tendency to break up into hair-like processes, and this fact suggests the view that the ridges are formed by the union of hairs or bristles which were originally scattered over the surface of the pellicula, as are the tubercles of *Platydactylus*.

The disposition of the ridges on the scales of *Lacerta* is subject to some variation in different regions. On the dorsal scales of the trunk they are arranged concentrically around the summit of the scale, the long slope being directed toward the base of the scale. On the large rectangular ventral scales the ridges are generally transverse in direction, with the long slope directed forward. In some cases the ridges bend backward near the lateral margin of the scale and run parallel with the edge, thus throwing the long slope toward the trough between the scales. On the shields of the head the general disposition of the ridges is similar to that just described, but the bending of the ridges at the lateral margins of the scale is very pronounced and constant. Moreover, at the posterior margin of the shield a similar change of the relations of the ridges is to be observed, the ridges bending through 180° in order to throw the long slope toward the trough between the shields. This arrangement is evidently a modification of that which occurs on the ordinary scales.

In the trough between the scales the ridges of the pellicula become irregular and correspond to the borders of the cells of the stratum internum. This relation suggests the kind of sculpture which occurs in the *Iguanidæ*.

In this family, as described by Blanchard, 80, the surface of the pelli-

cula is divided into more or less hexagonal areas, which correspond to the cells of the stratum internum. In *Phrynosoma* I find these areas separated by well-marked ridges, which bear conspicuous prickles at the angles of intersection. A similar sculpture has also been observed in *Moloch*, *Sceloporus*, and *Agama*. In *Phrynosoma* the hexagonal areas have an average diameter of about 22μ and the prickles may reach a height of 8μ . In this form the prickles show their strongest development on the ordinary scales, while they are small or wanting on the "horns" and large dorsal spines, where they would probably hinder exuviation. On the ordinary scales this difficulty is avoided by the inclination of the prickles, those which lie on the slopes of the scale being perpendicular to a plane passing through the base of the scale, so that all of the prickles on a given scale become more or less parallel in direction. A similar adjustment is to be observed also in the case of the ridges. Those which run parallel with the periphery of the scale become unsymmetrical, with the long slope directed toward the base of the scale.

The different accounts of the exuviation process, as described by Leydig, 72, Cartier, 74, Todaro, 79, Batelli, 79, and Blanchard, 80, disagree in regard to certain details and I shall not attempt a reconciliation here. The following facts, however, are well established. In its early stages exuviation is a purely physiological process which is characterized by the development of a new stratum corneum below the old layer. As a part of this growth a new pellicula is formed,—an impervious layer which lies next to the old stratum corneum. Owing to its development the old stratum is isolated and cut off from its supply of moisture and nourishment. Gradual dessication follows and the old stratum corneum is then ready for the second, or mechanical, stage of exuviation.

This stage probably begins with the development of the prickles or ridges of the new pellicula, which break the contact of the old stratum corneum with the new layer. The actual removal is then effected by the ordinary movements of the body or by other mechanical means. It may be added that the disposition of the ridges and prickles of the new pellicula is such as to facilitate the removal of the old stratum corneum.

In the earlier stages of exuviation an important part is played by the blood and lymph-vessels which convey nourishment to the cutis and to the Malpighian layer of the epidermis. Such vessels are abundant in the subcutaneous layer, whence the arteries run directly to the papillæ and break up into capillaries. The veins and lymph channels which drain the papillæ run for the most part beside the arteries.

Hyrzl, 38, p. 383, has recorded the observation that the number of

blood-vessels in the præ-ocular curtain of snakes increases greatly at the time of moulting, presumably as an aid to exuviation. If this is true, it is possible that a similar development may occur in the ordinary skin, and their occurrence might be expected also in the lizards.

Such a growth of new capillaries offers a field for the application of one or both of the following theories: (a) According to Thoma, 96, the "increase of blood-pressure in the capillary areas leads to the formation of new capillaries." Loeb, 93, on the other hand, claims that "Die Abgabe von Aesten ist bestimmt durch innere Ursachen in den Zellen der Gefässwände oder durch Reizursachen, die von der Umgebung ausgehend, diese Zellen treffen."¹⁰ The physiological processes which occur in the skin, particularly in the epidermis, during the early stages of exuviation may act as such an external stimulus and lead to the formation of new capillaries.

c. EXUVIATION BY MEANS OF BLOOD-PRESSURE.

Thus far in the description of the moulting process it has not seemed necessary to invoke the aid of a special mechanism for the removal of the old stratum corneum. Indeed, in the case of the trunk and limbs such a mechanism would seem to be superfluous. We may ask, therefore, what is the need of such a mechanism in the head? In answer it may be said:

(a) The exuviation of the head is the most difficult part of the entire moulting process. This is due, first, to the rigidity of the skull preventing those movements which assist in the moulting of other parts; second, to the close attachment of the skin to the lips and to the openings of the sense organs.

(b) The exuviation of the head is the most urgent part of the entire process. In the later stages of moulting, as soon as the old stratum corneum begins to separate, it becomes a menace to sight, hearing, and touch, and its prompt removal from the neighborhood of the sense organs is absolutely necessary in order that they may retain their normal efficiency. The difficulties and dangers of this stage might evidently be reduced to a minimum by the seclusion of the moulting animal and by hastening the process of exuviation. If the animal does not conceal itself it is exposed to the attacks of enemies, if it depends on concealment alone, it may suffer from lack of food. In either case, therefore,

¹⁰ For a critical review of these histo-mechanical theories, see Mall, *Am. Journ. Anat.*, Vol. V, pp. 231-253.

the circumstances seem to demand special facilities for hastening exuviation in the region of the head. This demand has been met by the development of the moulting mechanism.

This mechanism assists in exuviation both in a physiological and in a mechanical way.

1. **PHYSIOLOGICAL EFFECTS.**—The high blood-pressure which distends the capillaries, veins, and sinuses of the head, produces at the same time extensive movements of the lymph. In the deeper parts of the head the swelling blood-vessels tend to expel the lymph from the tissues and force it into the channels which lead from the head. Near the skin and mucous membranes, on the other hand, a large part of the lymph is forced toward the free surfaces; it fills the subcutaneous lymph-spaces, the smaller spaces of the cutis and the great sinuses, like those of the eyelids. From these spaces a more liberal supply of lymph invades the epidermis, enters the intercellular passages of the Malpighian stratum and penetrates still farther, by diffusion, into the more superficial strata. Such effects would apparently follow from the enlargement of the blood-vessels alone, but they are probably augmented by a more rapid transudation which increases the amount of lymph in the tissues.

The physiological significance of these lymph movements is evident. A richer supply of lymph to the epidermis means a more rapid metabolism and the acceleration of the processes of growth which prepare the way for the mechanical stage of exuviation. Moreover, the effects are probably not limited to the period of high blood-pressure alone, but continue to be felt after normal conditions are restored, perhaps until the moulting mechanism is again set in motion and another active change of the lymph is inaugurated.

These physiological effects are naturally felt in all parts of the head. They are especially important in the earlier stages of exuviation, but they also tend to hasten the moulting of belated parts after the removal has actually begun.

2. **MECHANICAL EFFECTS.**—The moulting mechanism facilitates exuviation in a mechanical way by causing enlargement of the head. In all the soft parts,—in the individual scale with its capillaries, and in larger areas covering the superficial veins and sinuses—the skin is more or less stretched by the swelling blood-vessels. The lymph-vessels also contribute to the development of this condition, which reaches its maximum in the orbital region, where the wrinkled skin is smoothed out by blood-pressure in the sinus orbitalis, while the eyelids themselves are swollen by the distension of the lymph-sinuses which they contain. As a result

of these processes the old inelastic stratum corneum is gradually separated from the more flexible and elastic new layer.

In the region of the external naris exuviation is effected especially by changes in the spongy body of the nasal vestibule (Figs. 2, 3, and 5, Plate II). The description given on pp. 68-70 indicates the variety of movements which has been observed here. These movements assist also in the removal of the stratum corneum of the vestibule itself, which is lined by the infolded epidermis. Since the spongy body of the nasal vestibule is under local control, these moulting movements may be executed also without simultaneous swelling in other parts of the head. The same movements of the spongy body are used for the removal of foreign substances which may enter the vestibule.

The mechanical stage of exuviation has not been followed from beginning to end and it is uncertain what the typical order of events may be, or if such an order exists. It is not improbable, however, that the actual removal of the old stratum corneum begins in the region of the orbit, where the great distension of the sinus orbitalis affects both the lids and the adjacent parts. After a beginning has been made each new movement adds more territory to that already gained, while between periods of high blood-pressure the process is hastened by scratching the head with the foot, by rubbing against a foreign body, perhaps, also, by licking the lips (*Lacerta*). In these different ways the old stratum corneum is gradually loosened and thrown off.

According to von Fischer, 82, some lizards enter water freely at the moulting periods and thus facilitate the removal of the exuviæ. *Trachydosaurus asper*, for example, is said to remain quietly immersed for some time. In the case of those lizards which have the habit of sunning themselves, the moulting is accelerated by such exposure and is made more difficult, or prevented altogether, by keeping the animals away from the sun.

In healthy animals exuviation occurs at somewhat regular intervals so long as external conditions (food supply, temperature, and moisture) are favorable. According to Knauer, 79, the lizards of Austria moult, under favorable conditions, every month. Under adverse conditions the intervals become longer and in sickly animals the exuviation is omitted altogether. A corresponding variation occurs also in regard to the length of time required to complete the moulting process, which may be accomplished in two days by a healthy *Lacerta*, while unfavorable conditions may lengthen the period to more than a week.

The above facts indicate the close relation of exuviation to the general well-being of the lizard. In the head region this relation is sufficiently important to warrant the development of a special mechanism to expedite the removal of the old stratum corneum. But the moulting of other parts is no less important in the long run. On this point Knauer, 79, p. 496, says:

“Unschwer kann man sich überzeugen, dass der Hautwechsel bei Lurchen und Kriechthieren durchaus kein nebensächlicher Akt ihrer Lebensthätigkeit, vielmehr ein ganz unerlässlicher Vorgang im Lebensprozess dieser Thiere sei, einerlei, ob nun Verhinderung der Häutung die Ursache des bald eintretendes Todes oder die Consequenz vorhergegangener Störung der eigentlichen Lebensthätigkeit ist.”

II. DESCRIPTION OF A SWELL MECHANISM IN THE HEAD OF OPHIDIA.

A. MUSCULUS CONSTRICTOR VENÆ JUGULARIS INTERNÆ.

In *Tropidonotus natrix* the special mechanism for elevating the blood-pressure in the head includes a single muscle, the *m. constrictor venæ jugularis internæ* (*m. c. j. i.*, Text Figs. 12 and 13), which is located at the point where the vena mandibularis enters the jugular vein. In the snake the muscle has no relation to the skeletal parts; it consists of striated fibers, chiefly circular or spiral in direction, but with an irregular layer of longitudinal fibers imbedded in the wall of the vein inside of the spiral fibers. In a specimen with a total length of 59 cm. the muscle envelops the jugular vein for a distance of about two millimeters, about one-half of which lies in front of the mouth of the vena mandibularis. The middle portion of the muscle includes five layers of spiral fibers, in addition to the longitudinal fibers.

The muscle surrounds also the terminal parts of the veins which enter the vena jugularis interna at this point: vena mandibularis, vena oesophagea, and vena cervicalis. On the vena mandibularis it extends forward beyond the mouth of the vena maxillaris and envelops also the posterior part of the latter vein.

Different physiological conditions of the *m. constrictor jugularis internæ* have been observed in the different specimens used in the preparation of this description. In one individual the jugular vein is closed, the muscle fibers are much thickened and the entire muscle forms a compact band about the vein, whose walls are also thickened and folded. In

another specimen (Text Fig. 12) the vein is dilated, its walls are thin and the muscle fibers are slender and more loosely arranged.

The *m. constrictor venæ jugularis internæ* has been observed in the European Ringelnatter (*Tropidonotus natrix*), in the black snake of the United States (*Zamenis constrictor*), in the sea snake (*Hydrophis Hardwickii*, Text Fig. 14), in a rattlesnake (*Crotalus adamanteus*), and in a species of *Helminthophis* from Jamaica. In all of these the muscle shows practically the same relations and structure.

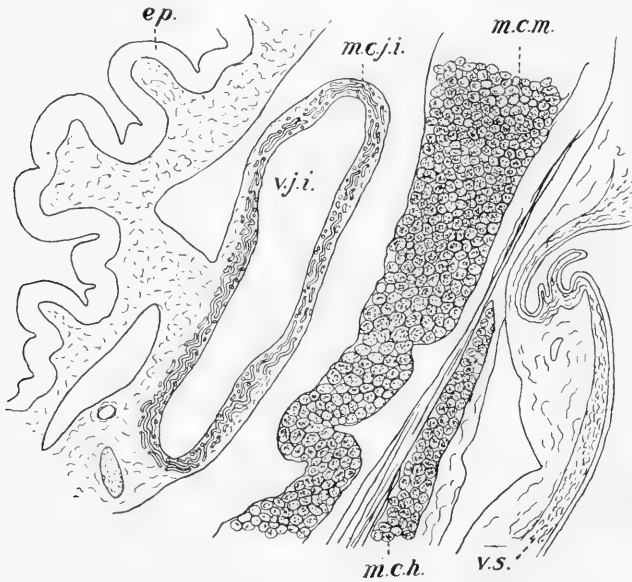


FIG. 12. Transverse section through the region of the *m. constrictor venæ jugularis internæ* of *Tropidonotus natrix*, right side. Specimen 55 cm. long. $\times 50$.

The section passes behind the mouth of the *vena mandibularis*.

ep., epithelium of pharynx; *m. c. h.*, *m. cervico-hyoideus*; *m. c. j. i.*, *m. constrictor venæ jugularis internæ* (the vein is dilated and the muscle fibers are relaxed and slender); *m. c. m.*, *m. cervico-mandibularis* (*sphincter colli*); *v. s.*, part of a ventral scale.

In *Tropidonotus* the *m. constrictor venæ jugularis internæ* is innervated by two small nerves, *nervi tumefactores capitis*, which arise from the vagus a short distance behind the inferior ganglion (*ganglion trunci vagi* of authors), where the vagus lies on the median side of the *vena jugularis interna*. One of these nerves enters the anterior border of the constrictor muscle above the jugular vein. The other nerve arises a

short distance behind its fellow and supplies the posterior part of the muscle, which it enters below the jugular vein, close to the junction of the vena mandibularis with the trunk vein.

The relations of the *m. constrictor venæ jugularis interna* of *Tropidonotus* are, in some respects, quite different from those of the constrictor muscle of the lizard. There is good reason to believe, however, that the



FIG. 13. Reconstruction of the *m. constrictor venæ jugularis interna* of *Tropidonotus natrix*, from a specimen 59 cm. long. Right side. $\times 22$.

The bundles of muscle fibers are spiral but the details are diagrammatic. The individual fibers are not indicated.

v. m., vena mandibularis; *v. m.x.*, vena maxillaris; *v. j. i.*, vena jugularis interna.

The vena œsophagea and vena cervicalis lateralis penetrate the posterior half of the muscle in *Tropidonotus* but they are not shown in the figure.

saurian muscle shows the more primitive relations and that the peculiarities of the muscle in *Tropidonotus* are due to general modifications of structure which occurred during the phylogeny of the Ophidia. The most important factor in modifying the relations of the muscle has probably been the development of the enormous gap and the distensible pharynx,

as a result of which the meeting points of the great cephalic veins have been shifted caudalward. This involved a corresponding change in the position of the muscle, which lost at the same time its skeletal attachments.

B. DISTENSION OF THE VEINS AND SINUSES.

No observations have been made on the flooding of the cephalic veins and sinuses of the Ophidia under natural conditions. The court plaster method has been employed with different species, but without success. On the other hand, artificial obstruction of the two venæ jugulares internæ produces protrusion of the eyes, enlargement of the sinus vestibuli nasi, and other phenomena of like nature. There is, therefore, no doubt in regard to the general effect of the contraction of the m. constrictor venæ jugularis internæ.

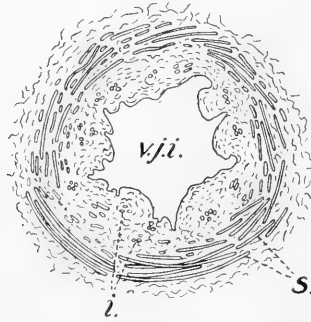


FIG. 14. Section of the m. constrictor venæ jugularis internæ of *Hydrophis hardwickii*. $\times 50$.

The inner fibers (*i.*) of the muscle are longitudinal in direction, the outer fibers (*s.*) are spiral. The vena jugularis interna (*v. j. i.*) is much constricted.

Whether this muscle is assisted by other muscles in raising the blood-pressure in the head is a question which must be left undecided. It is not impossible that an extraordinary blood-pressure may be produced by compression of the larger veins and sinuses after contraction of the constrictor muscle. Such an effect would apparently follow certain movements of the skeletal parts (palato-quadratum, maxillare, and the quadrato-squamosum), or it might be produced by the elevation of the floor of the mouth and tongue against the sinus palatinus and the vena maxillaris, both of which lie close to the oral mucous membrane. Higher blood-pressure, under the conditions mentioned, would also probably follow the contraction of the m. temporalis (m. parietali-quadrato-mandi-

bularis Hoffmann) whose anterior portion lies directly above the posterior extension of the sinus orbitalis. All of these movements, however, would tend to produce uniform elevation of blood-pressure throughout the head. In the snake there is no means of closing the outlets of the sinus orbitalis and creating local high pressure in the anterior head region.

The reduction of the flooded sinus orbitalis of the snake is effected chiefly by the muscles of the bulbus, but the process is facilitated by the general elasticity of the tissues. Owing to the absence of movable eyelids, the *m. depressor palpebrae inferioris* is wanting, as is also the smooth muscle of the orbit (*m. compressor sinus orbitalis*).

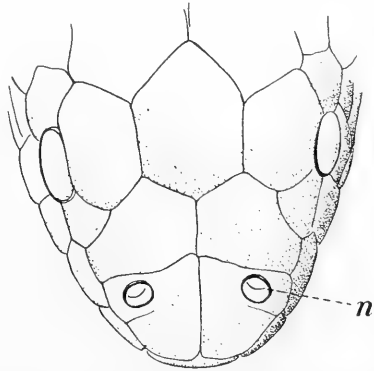


FIG. 15. *Hydrophis hardwickii*. The external nasal openings are closed by spongy tissue, chiefly by a swelling of the rostral wall of the nasal vestibule.

The flooding of the sinus vestibuli nasi produces effects which are easily observed in the sea snakes, where the external nasal opening may be closed by the spongy tissue of the nasal vestibule (Text Fig. 15 and Fig. 4, Plate II). Slight movements of a similar nature occur also in Colubroidea, but the spongy tissue is sparingly developed and the nasal opening cannot be closed in this group.

C. SIGNIFICANCE OF THE SWELL MECHANISM OF THE OPHIDIAN HEAD.

Concerning the ultimate significance of the swell mechanism of the Ophidian head there is little room for doubt, for the snakes have the same moulting habits as the lizards and there is apparently the same need of a moulting mechanism. In view of this fact and because of the close

phylogenetic relation of the two groups, it is reasonably certain that the Ophidia, as well as the Sauria, employ blood-pressure as an aid to exuviation. The great development of blood-vessels which Hyrtl, 38, found in the præocular curtain of snakes at the moulting time may be intimately associated with the high blood-pressure caused by obstruction of the vena jugularis interna.

The relative simplicity of the moulting mechanism in the Ophidia evidently corresponds to the character of the work to be done. On account of the union of the eyelids, and because of the absence of an external auditory depression, the moulting of the head is less difficult than in the Sauria. Moreover, in the snake the movements of the facial bones and suspensorium of the jaws must facilitate exuviation in a mechanical way and thus relieve the moulting mechanism of a part of its work. The details of the process must yet be verified, however, by a study of the moulting animal. As yet only the final stage has been observed.

This stage has been briefly described by Sharp, 90, who made his observations on two specimens of the American garter snake, *Eutania sirtalis* Linnaeus. These animals immersed themselves in water some time before the final stage began. The removal of the exuviae occurred immediately after the snakes left the water. By pressing the head into a narrow opening the skin was parted along the lips. The two flaps of skin were then turned backward above and below the head, and the animals crept forth, turning the skin inside out. The entire process occupied less than a minute and in one case the skin was removed without tearing. This is probably the typical mode of removing the old stratum corneum in the snakes.

Under normal conditions the moulting process is repeated at somewhat regular intervals. Thus, Gunther, 98, reports the following observations on the exuviation of Indian snakes kept in the Madras museum:

Python molorus moulted April 12, July 2, December 17.

Zamenis mucosus moulted April 22, May 18, June 15, July 8, August 18, September 5, October 5, November 7, December 14, 1896; January 17, February 27, 1897.

Tropidonotus stolatus moulted June 28, July 6, July 27, September 3, December 14, 1896; January 18, February 27, 1897.

Dendrophis pictus moulted April 2, May 6, June 26, July 27, October 29, 1896; died January 22, 1897.

III. THE SWELL MECHANISM OF THE TESTUDINATA.

A. THE MUSCULUS CONSTRICTOR VENÆ JUGULARIS INTERNÆ.

In the Testudinata the swell mechanism of the head includes a m. constrictor venæ jugularis internæ, which shows a strong and peculiar development. In *Emys europæa* the muscle begins directly behind the Eustachian tube and accompanies the vena jugularis interna under

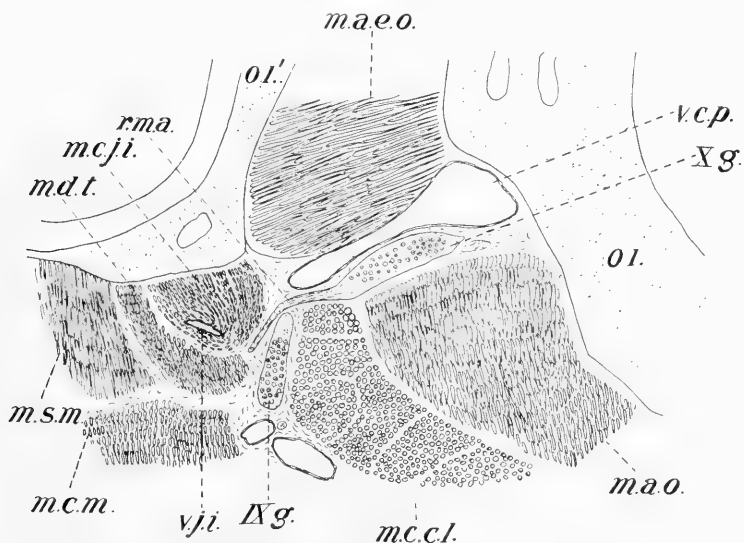


FIG. 16. Transverse section of *Emys europæa*, through the anterior part of the m. constrictor venæ jugularis internæ. $\times 13$. The section passes just behind the jugular foramen.

m. c. j. i., m. constrictor venæ jugularis internæ, anterior portion; *m. d. t.*, m. dilatator tubæ; *Ol.*, occipitale laterale of cranial wall; *Ol'*, occipitale laterale of parotic region; *r. m. a.*, ramus muscularis anterior of vago-accessorius (for *m. c. j. i.* and *m. d. t.*); *v. c. p.*, vena cerebialis posterior; *v. j. i.*, vena jugularis interna; *IXg.*, ganglion glossopharyngei; *Xg.*, ganglion superius vagi.

Other muscles represented are: *m. a. e. o.*, m. atlanto-epistropheo-occipitalis; *m. a. o.*, m. atlanto-occipitis; *m. c. c. l.*, m. collo-capitis longus; *m. c. m.*, m. cerato-maxillaris.

the floor of the tympanum and into the neck. The anterior half of the muscle (*m. c. j. i.*, Text Fig. 16) is composed chiefly of fibers which form loops below the vein, one arm of the loops lying median, the other lateral to the vein. The ends of these fibers are attached to the floor of the tympanic cavity above the vein. Some of these loops lie in a transverse plane but the majority are oblique, the median attachment being more posterior than the lateral attachment. This arrangement explains

the relation of the fibers in Text Fig. 16, where the fibers are attached chiefly on the median side of the jugular vein.

The anterior half of the constrictor muscle is very strong in *Emys* and the jugular vein is closely invested by the mass of muscle fibers. The function of this part of the muscle is indicated in Fig. 16, where the jugular vein is compressed by the contracted fibers.

The posterior half of the *m. constrictor venæ jugularis internæ* (*m. c. j. i.*, Text Fig. 17) occupies a position corresponding to that of the constrictor muscle of the lizard. The fibers of this part of the muscle arise in part from the parotic process (processus squamosus of Bojanus), in part from the tendon of the *m. sterno-mastoideus* (capiti-plastralis of

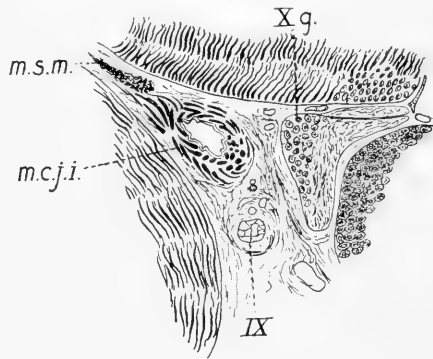


FIG. 17. Transverse section through the posterior part of the *m. constrictor venæ jugularis internæ* of *Emys europæa*. $\times 15$.

m. c. j. i., *m. constrictor venæ jugularis internæ*; the muscle here includes two rather regular layers of spiral fibers. *m. s. m.*, tendon of *m. sterno-mastoideus*; *IX*, glossopharyngeus; *Xg.*, posterior part of ganglion superius vagi.

Fürbringer). From their origin the fibers extend toward the *vena jugularis interna*, some passing above, some below the vein, about which they wrap themselves in a spiral direction running backward from their origin. Longitudinal fibers also occur, chiefly inside of the spiral fibers.

In a specimen of *Emys europæa* with carapace 16 cm. long the posterior part of the constrictor muscle envelopes the vein for a distance of about 2.5 mm. and includes seven or eight layers of fibers. The entire muscle has a length, measured on the vein, of 5.4 mm. The *vena cerebialis posterior* breaks through the posterior half of the muscle to reach the jugular vein.

The anterior part of the *m. constrictor venæ jugularis internæ* is closely related to the *m. dilatator tubæ* of Bojanus, 19-21, whose posterior

end (*m. d. t.*, Text Fig. 16) lies lateral to the vena jugularis interna, while its anterior end is inserted on the wall of the Eustachian tube directly ventral to the vein. In some cases the two muscles are not wholly separate, as fibers pass from one muscle to the other. The two muscles also receive their nerve supply from a common trunk. In view of its close relation to the constrictor muscle and to the vein it is possible that the *m. dilatator tubæ* may assist in obstructing the vena jugularis interna.

The constrictor muscle of the turtle is innervated by branches of a nerve which I designate *ramus muscularis anterior vagi* (*r. m. a.*, Text Fig. 16). This nerve arises from the anterior lateral portion of the ganglion radialis vagi, which lies median to the anterior part of the constrictor muscle. Near its origin the nerve divides into two unequal branches, the smaller of which, *nervus tumefactor capitis posterior*, bends directly caudad to enter the posterior part of the constrictor muscle. The larger nerve turns forward and divides again, one branch entering the *m. dilatator tubæ*, while the other, *nervus tumefactor capitis anterior*, supplies the anterior part of the constrictor muscle.

I have not attempted to determine definitely whether the fibers of the *nervi tumefactores capitis* belong to the vagus or to the accessorius. The roots of the two latter nerves form a close series and it is impossible to draw a line between them. By electrical stimulation of the roots in *Cistudo carolina* it has been found, however, that the fibers of the tumefactor nerves pass through the anterior members of the series; hence, they probably belong to the vagus of authors.

In addition to the forms mentioned, the *m. constrictor venæ jugularis internæ* has been demonstrated microscopically in the following species:

Kinosternon pennsylvanicum Gmelin.

Aromochelys odorata Bosc.

Aspidonectes spinifer LeSeur.

In all of these the constrictor muscle shows practically the same development as in *Emys*. The *m. dilatator tubæ* is also present and closely related to the constrictor muscle.

B. DISTENSION OF THE VEINS AND SINUSES OF THE TESTUDINATA.

The flooding of the cephalic veins and sinuses under natural conditions has not been observed in the Testudinata. The following experiments on the American speckled tortoise, *Clemys guttata* Schneider, are important, therefore, as indicating the probable nature and significance of the process:

Two small pieces of court plaster were applied to the left orbital

region, one directly in front of the anterior canthus, the other behind the posterior canthus. The plaster did not touch the eyelids themselves and their movements were not affected. At first the animal was shy and watchful and made no attempt to remove the plaster except by scratching with the foot. This movement was repeated several times, and on *both sides of the head*. Finally, after prolonged watching, the flooding of the sinus orbitalis was also observed. At first this occurred with the eyes open and alert, and the amount of protrusion was not large. Later, the sinus was flooded in a more marked way. As a preparation for this movement the animal became inattentive to its surroundings, then the eyes were closed and the head was drawn toward the opening of the shell. The enlargement of the orbital region then came on gradually and decreased in the same way, without any indication of a second stage. It is possible, however, that the regular movements were more or less modified or suppressed on account of the artificial conditions. After the reduction of the sinus orbitalis the eyelids were slowly wrinkled and distorted, as if by the contraction of a smooth muscle.

The response to this experiment agrees entirely with the character of the swell mechanism of the turtle. On account of the absence of the protrusor muscles (protrusor oculi and protrusor oculi accessorius), the outlet of the sinus orbitalis cannot be closed and it is impossible to develop local high pressure in the anterior part of the head. Whether the pressure can be increased by the action of other muscles, I am unable to say. Such an effect would apparently follow the contraction of the m. temporalis, which supports the posterior membranous wall of the sinus orbitalis. The elevation of the floor of the mouth against the flooded sinus palatinus would probably contribute to the same end by driving blood from that sinus into the sinus orbitalis. Under these conditions, however, the increase of blood-pressure must be more or less uniform throughout the head.

Corresponding to the absence of the protrusor muscles we find also a modification of the arrangements for reducing the flooded sinus orbitalis. The striated m. depressor palpebræ inferioris is wholly wanting in *Emys* and its place and function have been assumed, in part at least, by the smooth orbital muscle, m. compressor sinus orbitalis, which is very strongly developed in the turtle.

This muscle was first described by Bojanus, 19-21, Vol. I, p. 412, under the name musculus palpebralis. Stannius, 56, p. 172, recognized a m. palpebralis superior and a m. palpebralis inferior. Later, the two parts were again united by Hoffmann, 90, who, however, erroneously

described the muscle as a homologue of the striated m. depressor palpebrae inferioris of Weber, 77. The nature of the muscle explains the slow movements of the lower eyelid of the turtle; it also probably accounts for the very gradual reduction of the sinus orbitalis. Under ordinary conditions the muscle probably maintains a tonus which keeps the eyelids retracted and prevents distension of the sinus orbitalis. The elevation of the lower lid in *Emys* seems to be due chiefly to blood-pressure, as is the case in the lizard. In *Emys* there is no special muscle for the elevation of the lower eyelid, such as Stannius, 54, Vol. II, p. 172, has observed in *Chelydra* and *Chelonia*.

C. SIGNIFICANCE OF THE SWELL MECHANISM IN THE HEAD OF TESTUDINATA.

The experiments with *Clemmys* seem to show that the distension of the cephalic veins and sinuses has the same significance in the turtle as in the lizard. They confirm the view which is naturally suggested by a study of structure, that the swell mechanism of the turtle is a true moulting mechanism. On the other hand, however, we are confronted by the fact that a typical moulting process does not occur in the Testudinata. According to Gegenbaur, 98, the stratum corneum of the turtle wears away gradually and is renewed in the same way from below. Under the circumstances, the retention of the moulting mechanism may, perhaps, be explained on the theory that the method of desquamation has not reached a state of perfection which renders the mechanism wholly superfluous in getting rid of the old stratum corneum. Until additional evidence shall prove the contrary, therefore, we may consider the swell mechanism of the turtle as a true moulting mechanism.

IV. ONTOGENY OF THE BLOOD SINUSES OF THE REPTILIAN HEAD.

The ontogeny of the blood sinuses of the reptilian head has been investigated by Grosser and Brezina, 95, who find that the sinus orbitalis is formed by the gradual enlargement of a system of capillaries and veins, chiefly the vena orbitalis inferior and its tributaries, all of which are gradually distended until the soft tissues are largely displaced and a continuous sinus is formed which surrounds all the organs of the deeper part of the orbit. In an embryo of *Lacerta agilis*, series 20, head 6.3 mm. long, the adult condition has already been attained.

The numerous smaller sinuses of the head, which represent all stages of enlargement from the dilated capillary to the sinus palatinus, have,

undoubtedly, been formed in the same way as the sinus orbitalis, the amount of the expansion being determined, more or less, by the nature of the tissues which surround the vessels.

The chief factors in this ontogenetic process are presumably the following:

1. Heredity.
2. The muscle mechanism which obstructs the vena jugularis interna and elevates the venous blood-pressure in the head.
3. The vaso-motor and cardio-accelerator mechanisms.

The relative importance of these different factors is difficult to determine, but it may be expressed in a tentative way by the order in which they are named. It is probable that hereditary influences are more potent in the earlier stages of sinus making, while the later development is due more largely to the activity of the muscle and vascular mechanisms.

Positive evidence in regard to the value of heredity as a direct cause of sinus formation must be looked for in the earlier embryonic stages in which the muscle mechanism is not functionally developed, but the material at my disposal is not sufficient for the determination of this point. The influence of heredity is not limited, however, to the direct formation of sinuses; it contributes, also, the conditions which make the ontogenetic development possible, the most important of which, perhaps, is a certain indifference to pressure, which serves as a protection for the brain and other delicate parts.

The activity of the muscle mechanism during the later stages of embryonic life is indicated by different physiological conditions observed in sections of several species. For example, in an embryo of the black snake, *Zamenis constrictor* (head 4 mm. long), the *m. constrictor venæ jugularis internæ* is much contracted, the vein on one side of the head being practically closed. The sinus orbitalis is already well developed. Similar conditions have been observed in an embryo turtle, *Aromochelys odoratus* (head 1.5 mm. long) and in *Sceloporus undulatus* (head 2 mm. long). On the other hand, in a specimen of *Lacerta agilis* (head 5 mm. long) the constrictor muscle is relaxed and the diameter of the vein is not reduced.

In all of the cases mentioned the fibers of the *m. constrictor venæ jugularis internæ* are more or less immature and the observed effects are due to precocious activity. The effect of such activity on sinus development is apparent.

V. DISTRIBUTION AND PHYLOGENY OF THE SWELL MECHANISM.

In the preceding pages I have described a peculiar swell mechanism in the head of certain reptiles, including representative species of Sauria, Ophidia, and Testudinata. This mechanism is used by the Sauria for moulting purposes, and it probably performs the same function in the other orders. I now wish to review the taxonomic relations of the species studied and to show, as far as possible, the character and distribution of the swell mechanism in the suborders and families of modern reptiles. Afterward, the phylogenetic relations of the different reptilian orders will be considered and an effort will be made to determine the phylogeny of the swell mechanism and its probable distribution among extinct orders.

A. DISTRIBUTION OF THE SWELL MECHANISM AMONG MODERN REPTILES.

The general characteristics of the swell mechanism has been determined in representative species of the following orders, suborders, and families of reptiles:

a. SAURIA.

1. RHIPTOGLOSSA.—*Chamæleontidæ*. *Chamæleon vulgaris* Cuvier. The m. protrusor oculi is present; the m. protrusor oculi accessorius and m. constrictor venæ jugularis internæ are wanting. Blood sinuses are well developed in the anterior part of the head and in the cranial cavity. Extracranial veins are little enlarged in the posterior part of the head.

2. PACHYGLOSSA.—*Agamidæ*. *Agama colonorum* Daudin, *Moloch horridus* Gray. The m. constrictor venæ jugularis internæ and m. protrusor oculi are present; the m. protrusor oculi accessorius is wanting.

Iguanidæ. *Anolis caroliniensis* Cuvier, *Sceloporus undulatus* Latreille, *Phrynosoma cornutum* Harlan.

The muscles are the same as in the Agamidæ.

3. NYCTYSAURA.—*Geckonidæ*. *Platydictylus mauritanicus* Linnaeus. M. protrusor oculi and m. protrusor oculi accessorius are present. The m. constrictor venæ jugularis internæ is wanting. The sinus orbitalis, sinus palatinus, and sinus vestibuli nasi show about the same development as in other forms. The veins of the postorbital part of the head are little enlarged, excepting those of the cranium and certain others that are connected with the cranial vessels.

4. THECAGLOSSA.—*Varanidæ*. *Monitor niloticus* Hassl. Three strong muscles are present: m. constrictor venæ jugularis internæ, m. protrusor oculi, m. protrusor oculi accessorius.

5. DIPLOGLOSSA.—*Anguidæ*. *Anguis fragilis* Linnaeus. Two muscles are present: m. constrictor venæ jugularis internæ and m. protrusor oculi.

6. LEPTOGLOSSA.—*Lacertidæ*. *Lacerta agilis* Linnaeus, *Lacerta muralis* Merr. The constrictor muscle and the m. protrusor oculi are present. The m. protrusor oculi accessorius is wanting.

Teiidæ. *Cnemidophorus sexlineatus* Linnaeus. This species has the same muscles as *Lacerta*.

7. ANNULATI.—*Amphisbenidæ*. *Rhineura floridana* Baird. The essential parts of the moulting mechanism, protrusor and constrictor muscles and the facial sinuses, are wanting in this form.

b. OPHIDIA.

1. EPANODONTA.—*Typhlopidæ*. *Helminthopsis* (*species?*). The m. constrictor venæ jugularis internæ is present. The protrusor muscles are both wanting. Corresponding to the rudimentary condition of the eyes, the sinus orbitalis is much reduced; other sinuses are abundant, especially in the anterior part of the head.

2. COLUBROIDEA.—*Natricinæ*. *Tropidonotus natrix* Gesner. The constrictor muscle is present; protrusor muscles are wanting. Blood sinuses are well developed.

Colubrinæ. *Zamenis constrictor* Linnaeus shows practically the same conditions as *Tropidonotus*.

3. PROTEROGLYPHA.—*Hydrophidæ*. *Hydrophis hardwickii* Gray has remarkable blood sinuses, especially in the anterior part of the head. The constrictor muscle is strong; other muscles are absent.

4. SOLENOGLYPHA.—*Crotalidæ*. *Crotalus adamanteus* Beauvais. The m. constrictor venæ jugularis internæ was easily found in a recently hatched specimen.

Viperidæ. *Vipera berus* Linnaeus has blood sinuses similar to those of *Tropidonotus*, indicating the presence of the m. constrictor venæ jugularis internæ. No attempt was made to find the muscle itself.

c. RHYNCHOCEPHALIA.

Hatteria (*Sphenodon*) *punctata* Gray. I have made sections of the nasal vestibule of this species and find the spongy tissue well developed. The sinus orbitalis has been described by Osawa, 98, as "ein grosses

Blut-sinus, welches einen grossen Theil des Orbitalbodens einnimmt." There can be no doubt, therefore, in regard to the presence of some mechanism for obstructing the outlet of the sinus.

d. TESTUDINATA.

1. TRIONYCHIA.—*Trionichida*.. *Aspidonectes spinifer* LeSeur. The m. constrictor venæ jugularis internæ is present in a late embryo.

2. CRYPTODIRA.—*Kinosternida*. *Kinosternon pennsylvanicum* Gmelin. A mature embryo was examined; m. constrictor venæ jugularis internæ is present.

Emydida. *Cistudo* (*Emys* Wagl.) *europæa* Schneider and *Terrapene carolina* have been examined. The m. constrictor venæ jugularis internæ is strong in both species.

The foregoing list includes representatives of seven suborders of the Sauria, four suborders of Ophidia, and two suborders of Testudinata. The occurrence of the separate muscles of the swell mechanism is as follows:

The m. constrictor venæ jugularis internæ is present in all species examined (Sauria, Ophidia, and Testudinata), excepting *Chamaeleon vulgaris*, *Platydactylus mauritanicus*, and *Rhineure floridana*.

The m. protrusor oculi is present only in the Sauria, in which it occurs in all forms examined, excepting *Rhineure floridana*.

The m. protrusor oculi accessorius is also limited to the Sauria, in which it has been found only in the *Nyetysaura* (*Platydactylus*) and the *Thecaglossa* (*Monitor*).

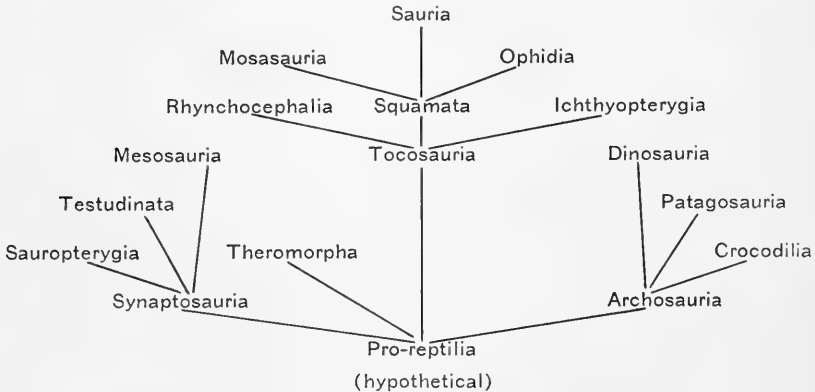
In the foregoing review we have found the swell mechanism present in all species examined, excepting a few aberrant forms. If this result is a fair index of the distribution of the mechanism, it probably occurs in all typical species and families of the Sauria, Ophidia, and Testudinata.

B. PHYLOGENY OF THE SWELL MECHANISM.

It is evident from the preceding observations that the swell mechanism is not confined to a few isolated reptilian forms, which might, perhaps, have produced analogous mechanisms along entirely independent lines. In spite of the somewhat divergent characteristics of the mechanism in the different orders and families of reptiles, we find certain parts, notably the m. constrictor venæ jugularis internæ, which are almost universally present. The homology of such parts is suggested both by their mor-

phology and physiology and has already been assumed in the earlier part of this paper. If this view is correct, we must conclude that the swell mechanism of the modern Sauria, Ophidia, and Testudinata has been inherited from common ancestors. It is, therefore, in order to show that this view is entirely in accord with the evidence furnished by phylogeny.

Paleontologists tell us that the lines of descent of the Sauria, Ophidia, and Testudinata meet in generalized forms of paleozoic age, from which all of the reptilian orders, both recent and fossil, have been derived. The probable relations of these orders, as interpreted by Fürbringer, 00, may be represented by the following diagram:



From these and similar views, which are advocated by Zittel, 87-90, Cope, 98, Lydekker, 88, 89, Osborn, 04, and others, we may conclude that the swell mechanism was already fully developed in pro-reptilian forms which became the ancestors of all the various orders of reptiles. According to the evidence of paleontology these primitive reptiles were lizard-like forms, which were provided with a scaly skin and a thick epidermis. It is probable, also, that they moulted the stratum corneum in true saurian fashion, utilizing the swell mechanism to accelerate the process.

If the foregoing views are correct, the origin of the moulting mechanism must be sought among the ancestors of the pro-reptilia. It is reasonably certain that the latter were descended from amphibians, probably from the Stegocephala, from which, also, they inherited the moulting habit. We may, perhaps, assume that the demand for such a mechanism first arose in that transition period when these an-

cestors gave up the aquatic habits and the glandular skin of the amphibians and began to develop a thick protective epidermis which is characteristic of typical reptiles. As a result of these changes exuviation became difficult, especially in the head region, with its rigid skull and numerous openings. To overcome the difficulty the moulting mechanism was developed, probably by the use of a branchial muscle which had become more or less superfluous under the new conditions of life. This muscle was endowed with a new function and became the *m. constrictor venæ jugularis internæ*.

Since the possessors of this moulting mechanism were ancestral to several orders of reptiles which have become extinct, it is not improbable that the mechanism existed in the latter, as well as in modern forms. According to Zittel, 87-90, the skin of these extinct reptiles, even that of the Ichthyopterygia and Sauropterygia, was relatively thick, and often provided with a horny epidermis. There is, therefore, reason to believe that the moulting mechanism was a necessity in these groups, as well as in others.

In the pro-reptilia the moulting mechanism probably included a single special muscle: the *m. constrictor venæ jugularis internæ*. But with the differentiation of separate orders came a demand for a more effective mechanism, especially in certain groups. In the Testudinata greater efficiency of the mechanism was secured, apparently, by a stronger development of the constrictor muscle itself. The improved mechanism has met the needs of this group of sluggish animals.

In the modern Ophidia the moulting mechanism shows a very simple condition, but it is doubtful if this condition is a primitive one, for since the Ophidia are descended from lizard-like forms, it is probable that the moulting mechanism of the snake has been modified in correlation with other changes which have rendered the exuviation less difficult. In the Colubroidea and Solenoglypha these changes include the union of the eyelids and the development of great mobility of certain bones of the head.

The highest form of the moulting mechanism has been developed among the Sauria, in which the *m. constrictor venæ jugularis* is assisted by one or two other muscles which are especially concerned in the exuviation of the anterior head region. The most primitive conditions, apparently, in this order are to be found in those forms which retain the second epibranchial cartilage. This cartilage gives attachment to the median part of the *m. constrictor venæ jugularis internæ*, which thus, in addition to its newly-acquired function as a constrictor, still exercises the

more primitive function of a branchial muscle. Associated with this primitive relation of the constrictor muscle, we find a single accessory muscle: the *m. protrusor oculi*. In other forms the same muscles are present but the epibranchial cartilage is wanting and the constrictor muscle is more strongly developed (*Phrynosoma*). In a few forms the moulting mechanism becomes still more efficient by the addition of a *m. protrusor oculi accessorius* (*Monitor*).

The complicated moulting mechanism of the *Sauria* is associated with movable eyelids and an external auditory depression, both of which render the moulting more difficult. At the same time we find great activity and intelligence demanding the prompt removal of the *exuviae*. In response to such demands, apparently, the moulting mechanism of the *Sauria* has come into existence.

The absence of the *m. constrictor venæ jugularis internæ* in *Chamæleon* and *Platydactylus* is undoubtedly a result of degeneration. In *Platydactylus* such a result is probably to be correlated with the union of the eyelids and the consequent removal of one of the chief difficulties of *exuviation*. In *Chamæleon* *exuviation* has been made easier by a close union of the head and trunk and the disappearance of the external auditory depression, changes which give greater flexibility to the skin of the posterior region of the head. The absence of the entire moulting mechanism in *Rhineura* is apparently to be explained, in part, by the loss of eyes and external auditory depression, in part, by the burrowing habit, which doubtless facilitates the removal of the old *stratum corneum*.

Degeneration of the moulting mechanism on a larger scale has probably occurred in the *Crocodylia*. The only surviving portion of the mechanism in this group seems to be the spongy body of the nasal vestibule, a structure of purely local importance. The loss of the major part of the mechanism is to be explained in this case by a radical change in the mode of renewing the *stratum corneum*. According to Gegenbaur, 98, the epidermis of the *Crocodylia* shows the same characteristics that we find in higher forms, the superficial strata wearing away gradually, while they are renewed in the same way from the *stratum Malpighii*.

A history similar to that just outlined might probably be written of the mammals. This group is probably descended from reptilian or pro-reptilian forms which were provided with the moulting mechanism. It is a matter of special interest, therefore, that modern mammals are furnished with a spongy body which is similar, both in structure and position, to that which occurs in the nasal vestibule of reptiles. The conclusion is almost irresistible that the two structures are homologous; in other words,

the spongy body of the higher forms is a relic of the moulting mechanism of reptile-like ancestors. The survival of the spongy body in the higher group, and also in the Crocodylia, may be easily explained by the development of arrangements for local control. It will be remembered that we found abundant evidence of such control of the spongy body in the lizards.

C. PHYLOGENY OF THE SINUSES.

The phylogeny of the venous sinuses of the reptilian head is indicated, in a general way, by their ontogeny. Somewhere along the line of descent of the modern reptiles ordinary veins and capillaries were enlarged to form the sinuses of the swell mechanism. The chief factors in this phylogenetic development were presumably the following:

1. The muscle mechanism which raised the blood-pressure in the cephalic veins by obstruction of the vena jugularis interna. At the time the sinuses were formed this mechanism probably included a single special muscle, the *m. constrictor venæ jugularis internæ*.

2. Cardio-accelerator and vaso-motor mechanisms, which increased the flow of blood to the head and thus contributed to the elevation of the venous blood-pressure.

3. Heredity.

The most important of these factors was undoubtedly the muscle mechanism. Comparison of living species, such as *Lacerta* and *Phrynosoma*, shows that the abundance and size of the sinuses correspond directly to the efficiency of the constrictor muscle, and this was probably true, also, in all stages of the phylogenetic development. When, for any reason, the muscle mechanism dwindled and disappeared, the sinuses disappeared also. This explains the absence or reduction of the sinuses in *Platydactylus*, *Chamæleon*, and *Rhineura*, in which the muscle mechanism for elevating the venous blood-pressure has been partly or wholly lost.

VI. COMMENT.

1. The swell mechanism of the lizards offers a partial solution of the problem concerning the ejection of blood from the orbit of the "horned toad," *Phrynosoma*. The sinus orbitalis forms a suitable reservoir for the reception of the blood to be ejected. This sinus may be filled with blood by contraction of the *m. constrictor venæ jugularis internæ*. The *m. protrusor oculi* could probably furnish sufficient force to cause the ejection of the accumulated blood, but if necessary, this muscle might be assisted by the smooth muscle of the orbit, *m. compressor sinus orbitalis*.

The participation of all of these muscles must be demonstrated, however, by a study of the ejection of blood under natural conditions.

Another undetermined point is the location of the opening through which ejection occurs. A suggestion in regard to this point has been obtained by manipulation. In vigorous specimens I have been able, in several cases, to force an ejection by compression of the two venæ jugulares internæ and elevation of the upper eyelid. The blood, in these cases, escaped from the membrana nictitans, which was thrown outward by the high blood-pressure in the sinus orbitalis. After the eye was restored to its normal condition there was no marked evidence of injury—no more than is to be observed when ejection occurs under normal conditions. A microscopic examination of specimens treated in this way showed an opening formed by rupture of the outer wall of the sinus membranæ nictitantis. Whether the location of the opening is the same under natural conditions, must yet be decided. In a specimen observed by Hay, 92, p. 376, the blood “was shot backward and appeared to issue from the outer canthus.” However, if the opening is in the membranæ nictitans, its position with reference to the outer lids would be variable.

I had hoped that the court plaster method employed in the investigation of the moulting mechanism might furnish an opportunity to study the mode of ejection, but up to the present time this method has failed to cause an ejection, although the distension of the sinus orbitalis has been frequently observed in *Phrynosoma*.

The failure of this method to induce ejection of blood would seem to oppose the idea of Stejneger, that the ejection occurs only during the moulting period, presumably as an aid to exuviation (see Hay, 92).

2. Some of the facts mentioned in this paper throw light on the question concerning the function of the spongy tissue of the nasal cavity of mammals. In man this tissue is well developed in that part of the mucous membrane which covers the inferior turbinate bone.

The most plausible theory hitherto suggested in regard to the function of this tissue is held by those who believe that the rich vascularization of the mucous membrane is necessary to give proper humidity to the inspired air and to raise its temperature. To this theory objection has been raised by Arviset, 87, on the ground that such a function could be best performed by a rich network of capillaries near the surface of the mucous membrane, while it would not explain the presence of the deeper sinuses of the spongy tissue. Arviset concludes that if the spongy body gives warmth and moisture to the inspired air, such a function must be accessory.

In view of Arviset's objection it seems necessary to admit that the foregoing theory fails to furnish a complete explanation of the problem under consideration. I wish, therefore, to propose a new theory which has been suggested by my studies on the reptiles. These cold-blooded, hibernating animals certainly do not require a special mechanism to elevate the temperature of the inspired air. It is also improbable that the stratified squamous epithelium of the nasal vestibule permits the escape of any considerable amount of moisture, which, moreover, would be readily supplied by the numerous glands of the nasal cavity. In the lizards and snakes the chief function of the spongy tissue of the nasal vestibule is to protect the entrance to the true olfactory chamber and lungs, either by closing the external naris, so as to exclude foreign bodies, or by facilitating the removal of any obstruction, whether it be the old stratum corneum or some foreign object. In the mammals the spongy tissue is not used to close the nasal vestibule nor to assist in exuviation, but there still remains the possibility that the respiratory passage may be blocked, either by secretions from the nasal cavity itself or by foreign matter. The removal of such an obstruction is doubtless facilitated by the alternate swelling and reduction of the spongy body of the inferior turbinate region. The origin of such a function is easily explained on the theory that the mammals have descended from reptilian ancestors, for if this is true, the spongy body has simply retained one of its primitive functions.

The survival of the spongy body in the nasal vestibule of crocodylians is probably to be explained by the exercise of a function similar to that just described for the apparently homologous structure in the mammals.

3. The occurrence of a mechanism for raising the venous blood-pressure in the head of reptiles suggests a few words in regard to the effect of such pressure on the delicate organs of the head. In the mammals the brain is particularly sensitive to blood-pressure and the intracranial sinuses are considered a special arrangement for preserving uniformity of pressure at all times. On this point, Foster, 94, p. 828, says:

"The channels for the venous blood of the brain are not veins but sinuses, not so much tubes for maintaining a uniform current as longitudinal reservoirs, which, while affording an easy onward path, can also be easily filled and easily emptied, and in which the blood can move to and fro without restriction of valves. This arrangement is correlated to the peculiar surroundings of the brain, which is not like other organs protected merely by skin or other extensible or elastic tissue, but is encased in a fairly complete inextensible envelope, the skull. As a consequence of this, when at any time an extra quantity of blood is sent from

the heart to the brain, room must be made for it by the increased exit of the fluids already present; for any pressure on the brain substance beyond a certain limit is injurious to its welfare and activity. Some room may be provided by the escape of cerebro-spinal fluid from the skull. But within the limits of the normal cerebral circulation the characteristic venous sinuses especially serve to regulate the internal pressure. . . . The injurious effects of too great a pressure on the brain substance are seen in certain maladies, where blood passing by rupture of a blood-vessel out of its normal channels remains effused on the surface of the brain or elsewhere, and by taking up the room of the proper brain substance leads, by 'compression,' as it is called, to paralysis, loss of consciousness, or death."

In the light of the foregoing account the head of the reptile presents a combination of apparently irreconcilable conditions. On the one hand we find intracranial sinuses designed apparently for the protection of the brain. On the other hand we see a mechanism for developing high blood-pressure, which must necessarily affect the brain as well as other sensitive parts. The fact that no serious consequences arise as a result of high blood-pressure might be explained on the assumption that the brain of reptiles is naturally less sensitive to pressure than is the more complex brain of higher forms. It is more than probable, however, that the brain of reptiles has acquired a certain immunity to pressure by reason of its long exposure to peculiar conditions.

Whatever the explanation may be, it is evident that the brain of lizards, snakes, and turtles is not at all sensitive to the pressure due to the distension of the veins of the cranial cavity. Hence, also, the intracranial sinuses lose their importance as a means of protecting the brain from the possible dangers of high pressure, for they fail at the time when the pressure is greatest. At such a time, they probably serve to equalize the blood-pressure within the cranial cavity or between the cranial cavity and other parts of the head. Their chief action, however, seems to be limited to ordinary conditions, when they regulate the intracranial blood-pressure, not as a means of protecting the brain, but in order to facilitate changes in the internal blood supply of that organ.

VII. SUMMARY OF PART SECOND.

1. The venous sinuses of the head of lizards, snakes, and turtles are associated with a mechanism which causes distension of veins and sinuses and thus produces intumescence and enlargement of the head.
2. In all three orders this swell mechanism includes a special muscle,

m. constrictor venæ jugularis internæ, which obstructs the chief efferent vessel of the head (vena jugularis interna). This muscle is located in the parotic region (lizard and turtle) or in the anterior cervical region (snake), at a point which controls the entrance of the more posterior cephalic tributaries of the vein, where the vein, under ordinary conditions, transmits nine-tenths of all the blood from the cranium, face, and jaws.

The constrictor muscle is innervated by fibers which come from the ganglion superius vagi (lizard and turtle) or from the ganglion trunci vagi (snake). In view of its innervation and general relations, the muscle has probably been derived from a branchial muscle of amphibian ancestors.

3. In the Sauria the swell mechanism includes also a m. protrusor oculi, the m. temporalis, and the bucco-pharyngeal muscles. The m. protrusor oculi is a hitherto undescribed muscle which lies at the origin of the vena jugularis interna from the sinus orbitalis. In contraction it obstructs the vein and at the same time presses against the membranous wall of the sinus. In some lizards a second protrusor muscle occurs, m. protrusor oculi accessorius, which lies directly behind the orbit, lateral to the m. protrusor oculi.

The protrusor muscles derive their nerve supply from the ramus mandibularis V.

4. The above-mentioned muscles usually contract in a certain sequence and produce two distinct stages of intumescence: a first stage with an average duration of about five seconds, and a second stage with a duration of about one-half second. The second stage is immediately followed by a stage of reduction. These three stages form a normal cycle of intumescence.

5. The first stage of intumescence begins with the contraction of the m. constrictor venæ jugularis internæ. The accumulation of blood is facilitated by relaxation of the orbital muscles and acceleration of the heart-beat, probably, also, by vaso-motor adjustments, including both dilation of the cephalic arteries and constriction of those leading to the posterior parts. During this stage all veins and sinuses of the head are rapidly filled and distended, the orbital region is protruded and more or less swelling occurs in other parts of the head.

6. The second stage of intumescence is marked by the contraction of the m. protrusor oculi, m. temporalis, and the bucco-pharyngeal muscles. The m. constrictor venæ jugularis internæ also maintaining its tonus. The result is a sudden increase of blood-pressure in the sinus orbitalis, the orbital region is more strongly protruded, and a wave of high blood-

pressure runs through all of the veins and sinuses which are tributary to the sinus orbitalis.

7. The stage of reduction is inaugurated by relaxation of the *m. constrictor venæ jugularis internæ*, the *m. protrusor oculi*, and the bucco-pharyngeal muscles. The reduction of blood-pressure is followed by a slowing down of the heart-beat, probably, also, by constriction of the carotids and dilation of the posterior arteries.

8. In the Sauria the swell mechanism is a moulting mechanism. It facilitates exuviation (*a*) in a physiological way, by accelerating the movement of the lymph and promoting the processes of metabolism; (*b*) in a mechanical way, by stretching the skin which covers the soft parts of the head.

The swell mechanism probably has the same function in the Sauria, Ophidia, and Testudinata.

9. In the Sauria the moulting mechanism may be set in motion by artificial stimuli, such as court plaster or mucilage, whose application to the head is followed by the same response that is observed under natural conditions.

10. The operation of the moulting mechanism is more or less reflex, but it may also be brought under voluntary control. If conditions are unfavorable for its operation the movements may be suppressed altogether.

11. The existence of the moulting mechanism in the Sauria and other reptiles is justified, first, because of the difficulties accompanying exuviation in the head; second, because of the demand for a prompt removal of the old stratum corneum from the openings of the sense organs.

12. In view of its distribution among modern reptiles, we may conclude that the moulting mechanism has been inherited from pro-reptilian forms which became the ancestors of all true reptiles, both ancient and modern. It is probable that the moulting mechanism was widely distributed among extinct reptiles.

13. The first development of the moulting mechanism probably occurred in transitional forms which were intermediate between amphibians and reptiles. The development was probably correlated with a thickening of the epidermis and the loss of cutaneous glands, the moulting process becoming more difficult on account of these changes.

14. The spongy tissue which occurs in the nasal vestibule of crocodylians and in the region of the inferior turbinate bone of mammals is probably a relic of the moulting mechanism of lower forms.

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GENERAL LIST OF ABBREVIATIONS.

PLATES I, II, AND III.

- A.*, atlas.
- a. f.*, arteria facialis (temporo-muscularis).
- B.*, bulbus.
- Bs.*, *Bs. p.*, basisphenoid bone.
- ep.*, epithelium of pharynx.
- Ep.*, second epibranchial cartilage.
- Ept.*, epipterygoid bone.
- Fr.*, frontal bone.
- g. n. e.*, glandula nasalis externa.
- g. r. f.*, ganglion of ramus frontalis ophthalmicus V.
- Imx.*, intermaxillary bone.
- J. o.*, Jacobson's organ.
- m.*, muscle fibers of spongy tissue of nasal vestibule.
- m. b.*, *m. bursalis*.
- m. c. c.*, *m. capiti-cervicalis*.
- m. c. j. i.*, *m. constrictor venæ jugularis internæ*.
- m. c. s. o.*, *m. c. s. o'*, *m. compressor sinus orbitalis*.
- m. d. m.*, *m. dorso-mandibularis*.
- m. d. p. i.*, *m. depressor palpebræ inferioris*.
- m. o. c. l.*, *m. occipito-cervicalis lateralis*.
- m. p. o.*, *m. protrusor oculi*.
- m. p. o. a.*, *m. protrusor oculi accessorius*.

- m. r. i.*, m. rectus inferior.
m. r. o., m. retractor oculi.
m. r. s., m. rectus superior.
m. t., m. temporalis.
Mx., maxillary bone.
N., nasal bone.
O. c., occipitale condyle.
Ol., occipitale laterale.
P. a., pila accessoria of chondrocranium.
Par., parietal bone.
pit., pituitary body.
Prf., præfrontal bone.
Ps., parasphenoid bone.
Pt., pterygoid bone.
Ptf., post-frontal bone.
Q., quadrate bone.
r. c. m., ramus communicans n. glossopharyngei cum n. maxillari.
r. d. p. i., ramus ad m. depressorem palpebræ inferioris.
r. c. e., ramus communicans externus n. glossopharyngei cum n. faciali.
r. c. i., ramus comunicans internus n. glossopharyngei cum n. faciali.
r. m., ramus mandibularis V.
r. p. f., ramus posterior VII.
r. v., pars ventralis of r. com. n. glossopharyngei cum n. maxillari.
S. i., subiculum infundibuli of chondrocranium.
Smx., septomaxillary bone.
S. n., septum nasi.
s., *s. o.*, sinus orbitalis.
Sq., squamosum.
s. s., sinus subnasalis.
s. t., spongy tissue of the nasal vestibule.
St., supratemporal bone.
s. V., secondary connection of the vena cerebialis media.
s. v. n., blood space of the sinus vestibuli nasi.
t., fibrous sheet connecting the two mm. protrusores oculi.
t', fibrous band by means of which the m. protrusor oculi inserts on the cartilaginous basis cranii.
T. m., tænia marginalis of chondrocranium.
T. p. m., tænia parietalis media.
thy., thymus gland.
v. c. p., vena cerebialis posterior.
v. j. i., vena jugularis interna.
v. l. s., vena labialis superior.
v. m., vena mandibularis.
v. mx., vena maxillaris.
v. n., nasal vestibule.
v. pt., vena pterygoidea.
v. s. m., vena suprasedalis media.
v. st., vena supratemporalis.
IX, X, XI, XII, cranial nerves.

EXPLANATION OF FIGURES.

PLATE I.

FIG. 1. Transverse section through the parotic process of *Phrynosoma*, showing the general relations of the m. constrictor venæ jugularis internæ. $\times 6$.

A., Atlas; *m. c. c.*, m. capiti-cervicalis; *m. c. j. i.*, m. constrictor venæ jugularis internæ; *m. d. m.*, m. dorso-mandibularis; *m. o. c. l.*, m. occipito-cervicalis lateralis; *O. c.*, occipital condyle; *Par.*, parietal bone; *Q.*, quadrate; *Sq.*, squamosum; *St.*, supratemporale.

FIG. 2. Musculus constrictor venæ jugularis internæ of *Phrynosoma*. Transverse section near Fig. 1. $\times 16$.

ep., epithelium of pharynx; *m. c. j. i.*, m. constrictor venæ jugularis internæ; the lumen of the vein is somewhat reduced by contraction of the muscle; *Ol.*, occipitale laterale; *St.*, supratemporale; *thy.*, thymus gland; *v. c. p.*, vena cerebialis posterior; *IX, X, XII*, cranial nerves.

FIG. 3. Transverse section of the m. constrictor venæ jugularis internæ of *Lacerta agilis*, showing attachment of the muscle. $\times 20$.

Ep., second epibranchial cartilage of Parker; *m. c. j. i.*, m. constrictor venæ jugularis internæ; *Ol.*, occipitale laterale; *r. c. e.*, ramus communicans externus n. glossopharyngei cum n. faciali; *r. c. i.*, ramus communicans internus n. glossopharyngei cum n. faciali; *r. v.*, pars ventralis of the ramus communicans n. glossopharyngei cum n. maxillari; *r. p. f.*, ramus posterior facialis; *St.*, supratemporal bone; *v. c. p.*, vena cerebialis posterior; *v. j. i.*, vena jugularis interna; *v. m.*, vena mandibularis; *IX, X, XI*, cranial nerves.

FIG. 4. Transverse section of head of *Lacerta agilis* through the posterior part of the m. protrusor oculi. $\times 10$.

a. f., arteria facialis (temporo-muscularis); *m. p. o.*, m. protrusor oculi; *m. t.*, m. temporalis; *Ps.*, parasphenoid bone, above which lies the basis cranii, formed by the united trabeculæ cranii of the chondrocranium; *Pt.*, pterygoid bone; *r. c. m.*, ramus communicans n. glossopharyngei cum n. maxillari; *r. d. p. i.*, ramus ad m. depressorem palpebræ inferioris; *r. m.*, ramus mandibularis V; *T. m.*, tænia marginalis of chondrocranium; *T. p. m.*, tænia parietalis media of chondrocranium; *v. j. i.*, vena jugularis interna; *v. pt.*, vena pterygoidea; *v. st.*, vena supratemporalis.

FIG. 5. Transverse section of *Lacerta agilis*, through anterior part of the m. protrusor oculi. $\times 25$.

g. r. f., ganglion of ramus frontalis ophthalmicus V; *m. b.*, m. bursalis; *m. p. o.*, m. protrusor oculi; *m. r. o.*, m. retractor oculi; *m. t.*, m. temporalis; *Ps.*, parasphenoid bone, above which lies the basis cranii, a cartilage formed by the union of the trabeculæ cranii; *r. d. p. i.*, ramus ad m. depressorem palpebræ inferioris; *S. i.*, subiculum infundibuli of chondrocranium; *s. V.*, secondary connection of the vena cerebialis media; *t.*, fibrous sheet which connects the two mm. protrusores oculi; *t'*, fibrous band by means of which the m. protrusor oculi inserts on the cartilaginous basis cranii; *v. j. i.*, vena jugularis interna.

PLATE II.

FIG. 1. Reconstruction from an adult *Monitor niloticus*, to show the relations of the *m. protrusor oculi*, *m. protrusor oculi accessorius* and *m. temporalis*, as viewed from a medial direction. $\times 8$. Somewhat diagrammatic.

B., bulbus; *Bs.*, basisphenoid bone; *Ept.*, epipterygoid bone (columella); *Fr.*, frontal bone; *m. c. s. o.*, *m. compressor sinus orbitalis*; *m. c. s. o'*, portion of *m. compressor sinus orbitalis* which enters the *membrana nictitans*; *m. d. p. i.*, fascia from which the *m. depressor palpebræ inferioris* takes its origin. In *Monitor* this fascia is separate from the fascia of the *m. protrusor oculi*. *m. p. o.*, *m. protrusor oculi*; *m. p. o. a.*, *m. protrusor oculi accessorius*; *m. t.*, *m. temporalis*; *P. a.*, *pila accessoria* of chondrocranium; *Prf.*, *Præfrontale*; *s. o.*, *sinus orbitalis*; *T. m.*, *tænia marginalis*; *v. j. i.*, *vena jugularis interna*.

FIGS. 2 AND 3. Transverse sections through the nasal vestibule of *Lacerta agilis*. Fig. 2 shows a section close behind the external nasal opening; Fig. 3, a section through the posterior part of the vestibule. $\times 23$.

C., cartilaginous nasal capsule; *Imx.*, *Intermaxillary bone*; *J. o.*, capsule of Jacobson's organ; *Mx.*, *maxillary bone*; *N.*, *nasal bone*; *Smx.*, *septomaxillary bone*; *s. t.*, *spongy tissue of nasal vestibule*; *s. t'*, *body of spongy tissue behind the external nasal groove, n. g.*, of Fig. 2; *v. l. s.*, *vena labialis superior*; *v. mx.*, *vena maxillaris*; *v. s. m.*, *vena suprasedentalis media*.

FIG. 4. Transverse section of *Hydrophis hardwickii* just behind the external nasal opening. $\times 22$.

J. o., Jacobson's organ; *m.*, bundles of smooth muscle fibers of the spongy tissue of the nasal vestibule; *Smx.*, *septomaxillary bone*; *S. n.*, *septum nasi*; *s. s.*, *sinus subnasalis*. It opens above into a still larger sinus from which the *vena maxillaris* takes its origin. *s. v. n.*, one of the blood spaces of the *sinus vestibuli nasi*; *v. mx.*, *vena maxillaris*; *v. n.*, *nasal vestibule*.

FIG. 5. *Phrynosoma cornutum*. Transverse section through the nasal vestibule close to the external nasal opening. In front of the section the vestibule bends laterad in the direction of the line *s. t.* $\times 21$.

g. n. e., *glandula nasalis externa*; *m.*, smooth muscle fibers of the spongy tissue of the nasal vestibule; *N.*, *nasal bone*; *Prf.*, *præfrontal bone*; *Smx.*, *septomaxillary bone*; *S. n.*, *septum nasi*; *s. t.*, *spongy tissue*. The particular body of tissue indicated by the line lies directly behind the external nasal opening.

PLATE III.

FIG. 1. Transverse section of *Phrynosoma cornutum* through the region of the *m. protrusor oculi*. $\times 11$.

The section is somewhat oblique, the right side being more anterior than the left.

Bs., *basisphenoid bone*; *Bs. p.*, *process of basisphenoid which gives rise to the m. protrusor oculi*; *m. b.*, *m. bursalis*; *m. d. p. i.*, *m. depressor palpebræ inferioris*; *m. p. o.*, *m. protrusor oculi*; *Ps.*, *parasphenoid bone*; *pit.*, *pituitary*

body; *Pt.*, pterygoid bone; *s. o.*, posterior prolongation of the sinus orbitalis; *v. j. i.*, vena jugularis interna.

FIG. 2. Transverse section through the posterior part of the orbit of *Monitor niloticus*, to show the relations of the *m. protrusor oculi* and *m. protrusor oculi accessorius*. $\times 11$.

Fr., frontal bone; *l.*, lymph sinuses; *m. c. s. o.*, *m. compressor sinus orbitalis*; it is represented only by a fascia under the bulbus in the region of the section; *m. d. p. i.*, *m. depressor palpebræ inferioris*; *m. p. o.*, *m. protrusor oculi*; *m. p. o. a.*, *m. protrusor oculi accessorius*; *m. r. i.*, *m. rectus inferior*; *m. r. o.*, *m. retractor oculi*; *m. r. s.*, *m. rectus superior*; *Par.*, parietal bone; *Ps.*, parasphenoid bone; *Pt.*, pterygoid bone; *Ptf.*, postfrontal bone; *s.*, sinus orbitalis.

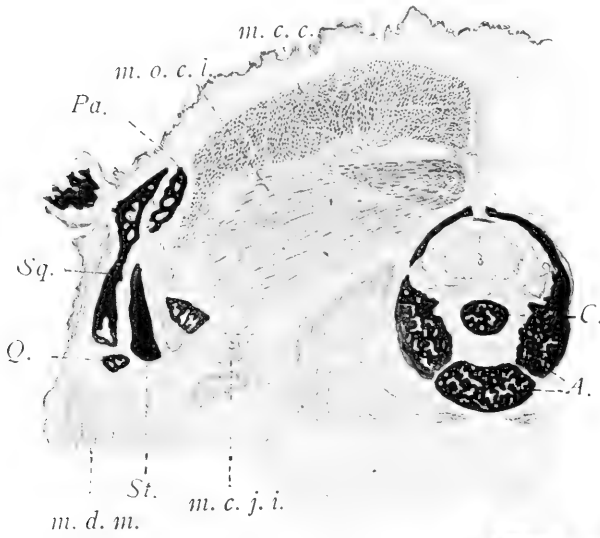


FIG. 1.



FIG. 2.



FIG. 3.



FIG. 4.



FIG. 5.

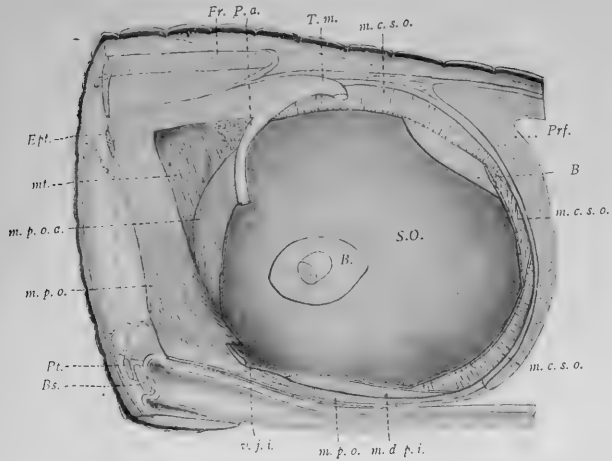


FIG. 1.

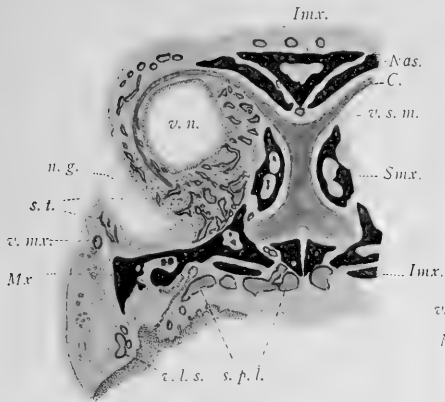


FIG. 2.



FIG. 3.

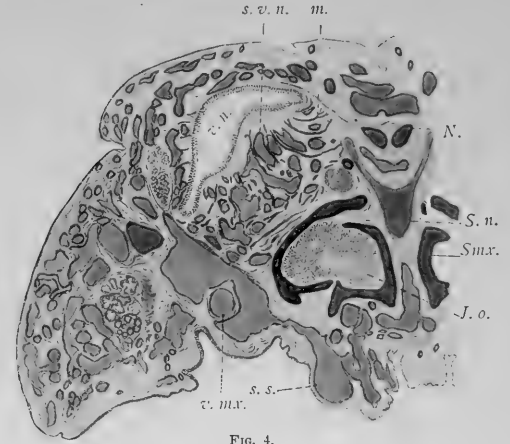


FIG. 4.

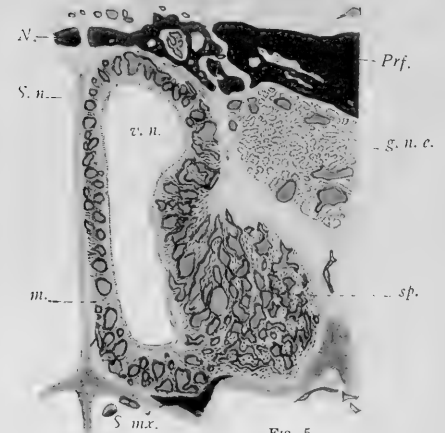


FIG. 5.



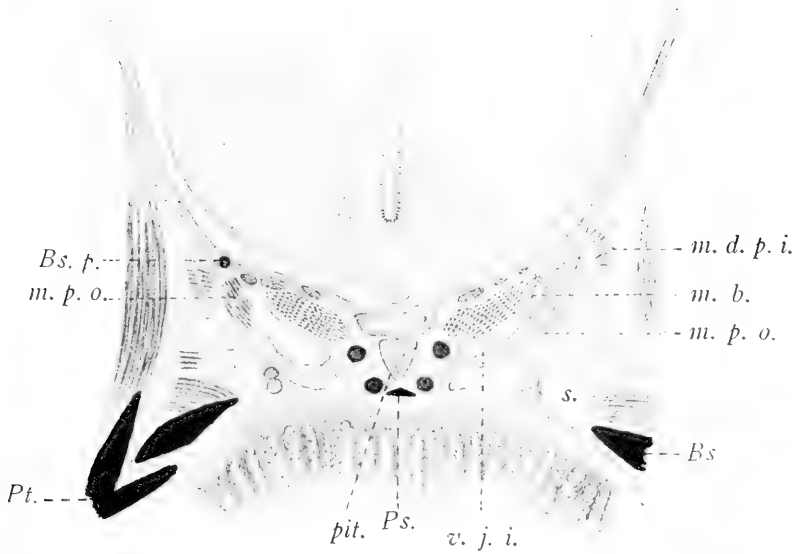


FIG. 1.

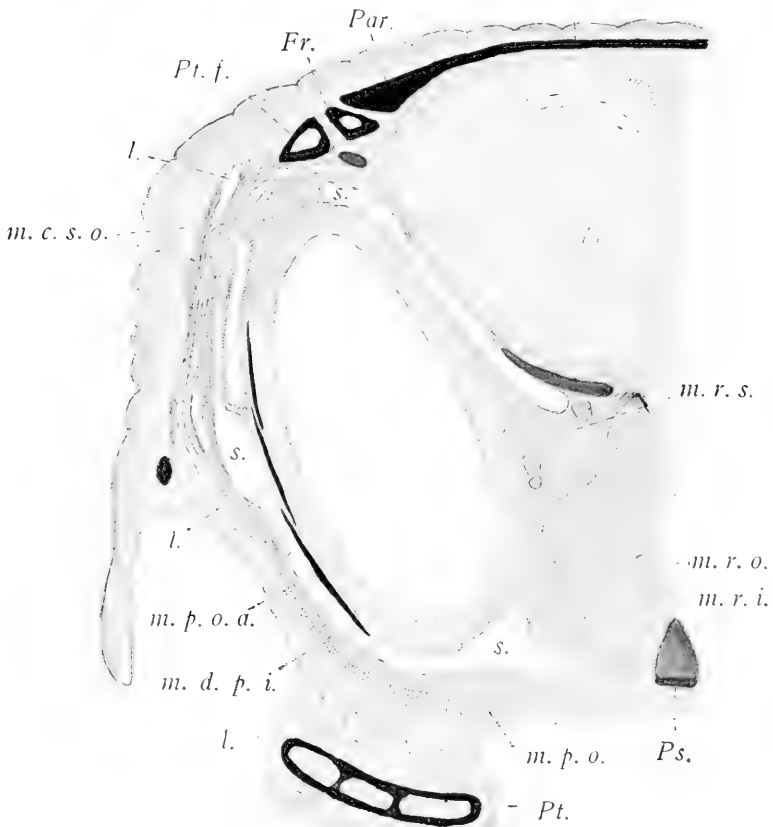


FIG. 2.

THE CORPUS PONTO-BULBARE—A HITHERTO UNDE-
SCRIBED NUCLEAR MASS IN THE HUMAN HIND BRAIN.

BY

CHARLES R. ESSICK,
Johns Hopkins Medical School.

WITH 12 FIGURES.

In the following paper I have described a ganglionic mass which can be seen overlapping the restiform body just caudal to the dorsal cochlear nucleus and forming a direct lateral process or extension of the ganglion mass of the pons. This structure was undoubtedly seen long ago by Clarke,¹ but he considered it to be an aberrant strand from the striæ medullares, and probably it had been seen by Arnold, who also described it as belonging to the strial system. The caudal tip, which is often prominent, has attracted the attention of various authors, and was particularly described by Retzius² and Henle³ under the name "Ligula" and "Ponticulus"; none of these observers, however, followed the structure in cross sections, and its significance was consequently overlooked. As far as could be learned, the following paper gives the first description of its whole form and for the first time demonstrates its connection with the ganglion mass of the pons. I have found that it is constantly present to a greater or less degree in all human brains and possesses definite characteristics which seem to me to warrant a name such as is herewith proposed—the corpus ponto-bulbare or ponto-bulbar body.

An adult formalin specimen, in which the structure was especially prominently developed, was photographed (Fig. 1) and after mordanting in a double chrome salt solution was imbedded in celloidin and cut in serial sections. The alternate sections were stained by the Weigert-

¹ Mr. J. L. Clarke: *On the Intimate Structure of the Brain.* Phil. Trans. London, 1868.

² Gustaf Retzius: *Das Menschenhirn*, Vol. I, Stockholm, 1896.

³ J. Henle: *Handbuch der Systematischen Anatomie des Menschen*, Vol. III, Part II, Braunschweig, 1879.

Pal method; the intermediate ones, for purpose of cell study, were stained by various cell stains, thionin having proved most satisfactory. A wax-plate reconstruction after the method of Born was made from this series showing the ponto-bulbar body and its relations to the adjacent cranial nerves, the cochlear nuclei, the trapezoid body, and the restiform body. A composite drawing of this model showing its relations to the surrounding region is represented in Fig. 12.

Through the kindness of Prof. Franklin P. Mall and Dr. Florence R. Sabin, I had the opportunity of making a macroscopical examination of fifty brains fixed in formalin, a description of which is included in



FIG. 1. Photograph of a human medulla with a well-marked corpus ponto-bulbare (c), transverse sections of which are represented in Figs. 3-11. Fig. 12 represents the same medulla reconstructed into a model and drawn in ventro-lateral view. $\times 2$.

this paper. They also placed at my disposal several series of both adult and new-born medullas which were valuable as a control over my own series. For these and many other courtesies on their part I take pleasure in using this opportunity to express my thanks.

GROSS APPEARANCE.

The structure, which I have designated as the corpus ponto-bulbare, constitutes a horn-shaped process of the pons which extends caudally, wrapping around the lateral side of the restiform body so as to end on its dorsal surface; forming, in part, the lateral boundary of the fourth ventricle. If one takes a brain from which the pia mater has been

stripped off, the ponto-bulbar body can be seen making its appearance on the ventro-lateral surface of the pons near the emerging root bundles of the trigeminal nerve and extending backward passing between the roots of the acoustic and facial nerves. Continuing backward ventral to the restiform body the main mass passes dorsal to the emerging glosso-pharyngeal roots, finally, assuming the dorsal position shown in Figs. 1 and 2. So that the ponto-bulbar body is superficial throughout its extent and its development usually throws the surface into a more or less well-marked fold which can be traced from its beginning on the ventro-lateral surface of the pons to its ending at the lateral wall of the fourth ventricle. As soon as these caudally directed fibers leave the pons it becomes evident that they are accompanied by a cellular mass which is itself distinct from the neighboring structures. This nuclear mass fused with the pons between the facial and acoustic nerves gains the dorsal surface of the medulla by passing around the restiform body caudal to the dorsal cochlear nucleus.

Out of fifty brain specimens which were examined macroscopically, it was found that nearly all showed clearly the ponto-bulbar body throughout its entire extent. About half showed a prominent ridge from its beginning on the pons to its ending at the lateral wall of the fourth ventricle. In all the brains examined, the ponto-bulbar body was visible macroscopically in some part of its course. The reason it is not seen throughout its whole extent in all of the specimens is not due to its absence, but to its retraction beneath the surface of the hind-brain.

At the point of its emergence on the pons its position, size, and shape vary considerably in different brains. The ponto-bulbar body appears first as small bundles of fibers which turn away from the transverse pons fibers proper somewhere median to the trigeminal nerve. Gradually these bundles converge to form a more or less compact ridge which curves backward just behind the fifth nerve and runs caudally almost at right angles to the other pons fibers, to pass between the facial and acoustic nerves. The eminence thus formed is generally single as far front as the fifth nerve and measures from 3-6 mm. in width; the extent to which it is raised above the pons surface varies from a sheet-like layer of fibers to a corded ridge. The manner in which the ponto-bulbar body sweeps out of the pons resembles somewhat the converging roots of the cauda equina; one specimen showed a distinct claw-like arrangement of fiber bundles. At times the fibers go to form two separate ridges which unite just cephalad to the facial nerve. The region from which these fibers turn

out from the pons extends from a point about 5 mm. in front of the fifth nerve to 4 mm. in front of the seventh nerve. An interesting variation to this general arrangement is found where fiber bundles encircle the trigeminal nerve or appear to come up out of the pons immediately behind it, and then take the regular course between the facial and acoustic nerves. Few specimens did not show this part of the ponto-bulbar body very plainly.

Although no reference is made to it, an admirable picture of the pontine part is given by Retzius⁴ in his Atlas, Plate IV, Fig. 1. On the left half of the brain (right side of figure) the eighth and seventh nerve roots can be seen just median to the flocculus. In front of these nerves a definitely corded ridge is present passing forward, median to the trigeminal nerve to be lost in the pons. The choroid plexus from the lateral recess hides the corresponding right part of the brain in the neighborhood of the seventh and eighth nerves, but the way in which the ponto-bulbar body arises from the transversely directed pons fibers, median to the root bundles of the fifth nerve, and then assumes a caudal direction, is particularly well illustrated.

By far the most constant part of the ponto-bulbar body, appearing on mere gross examination of brain, is the welt-like ridge which it forms ventral to the restiform body after it has passed between the facial and acoustic nerves. This portion is difficult to display in the whole brains preserved in formalin, because it necessitates removing the pia mater from the depression between the flocculus and the olive and, unless great care is used, this procedure takes with it the root bundles of the glosso-pharyngeal nerves. The part of the ponto-bulbar body which is then brought into view, extends about 6 mm. caudally as a prominent elevation having a width of 3 mm. in all specimens which were measured. We find it nearly always extending so as to surround the most cephalic of the glosso-pharyngeal roots. In this event, the appearance is that of a hill-shaped eminence with the stumps of the glosso-pharyngeal nerve emerging from the summit. On the left side of the brain illustrated by Retzius,⁵ Fig. 11, Plate XXXVIII, there appears the best illustration of this portion of the ponto-bulbar body. Here the hypoglossal nerve roots may be recognized as they emerge mesial to the olive. Lateral to the olive is a well marked ridge with five root bundles of the glossopharyn-

⁴ Gustaf Retzius: *Biologische Untersuchungen*, Neue Folge XII, 1905.

⁵ Gustaf Retzius: *Das Menschenhirn*, Vol. II, Stockholm, 1896.

geal nerve emerging from the summit. This ridge can be traced anteriorly as it passes between the facial and acoustic nerves and a very little part of the pontine portion may be seen just anterior to the seventh nerve as is indicated by vertically directed lines in contrast to the horizontally placed lines representing the direction of the pons fibers proper. Other figures, 9, 12, and 13, of the same plate, furnish good illustrations of this region.

At the point where it emerges below the lateral recess, we see it turning around the restiform body just caudal to the dorsal cochlear nucleus from which it is separated by a shallow sulcus. In some specimens it might easily be mistaken for the dorsal cochlear nucleus; but if one will carefully follow the cochlear nerve, the real dorsal cochlear nucleus can be made out in front of the attachment of the tela choroidea inferior, i. e., the dorsal cochlear nucleus lies in the lateral recess and is covered with ependymal cells; while the ponto-bulbar body, which often approaches the dorsal cochlear nucleus in elevation and extent, is situated more caudal, and makes a curve of greater radius around the restiform body to gain the dorsal surface of the medulla.

On the dorsal surface of the medulla, the ponto-bulbar body undergoes its greatest variation in different brains and, in fact, on the two sides of the same brain. Instead of the welt-like ridge which it presents in the more cephalic part of its course, it here spreads out into a flattened leaf-like structure overlapping the restiform body and the lateral part of the floor of the fourth ventricle. In its exact form it shows considerable variation. Of the fifty brains which were examined five of them presented the prominent mitten-like form 4.5 — 6.0 mm. wide with a free tip, projecting, as it were, out into the tela choroidea, as is seen in the Fig. 1. These would be called markedly well developed. On the other hand, in about 40% of the specimens this part of the ponto-bulbar body forms such a thin lamella that it is not noticeable macroscopically on at least one side of the brain. Between these two extremes there are all gradations. It may take the form of a single ridge usually 2 — 3 mm. in width; or may be divided into two finger-like ridges. In well developed cases the caudal tip may help to form the roof of the fourth ventricle, forming a free end to the edge of which is attached the tela choroidea. In the specimen photographed, Fig. 1, there was a tip of this kind 1.5 mm. long. The fact that the tela choroidea is attached to it, makes it a thing easily damaged in removal of the pia. A drawing of an average human medulla is represented in Fig. 2 and this gives the usual position and size which the ponto-bulbar body presents in a dorsal

view of the fourth ventricle. It may be seen turning around the restiform body caudal to the line of attachment of the tela choroidea along the lateral recess and projecting as a lamella into the roof of the fourth ventricle.

There are many excellent photogravures of this region in Retzius,^o in which the relations of the ponto-bulbar body to the restiform body and the fourth ventricle are brought out. In his Plate XXXV, Fig. 9 shows

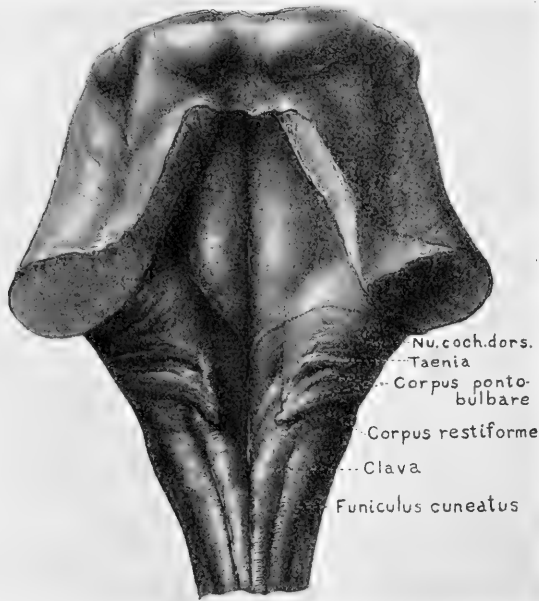


FIG. 2. Drawing of an average human medulla giving the usual appearance of the corpus ponto-bulbare caudal to the dorsal cochlear nucleus. $\times 2$.

a doubled ridge, above described as "finger-like," on the right side extending antero-lateral from the base of the detached tania (labeled "t"). A similar though not so well marked arrangement is shown in Plate XXXVI, Fig. 2. Figure 4 of Plate XXXVII gives the best illustration of the whole dorsal extent of the ponto-bulbar body because the pia has been completely removed from the specimen. It may be recognized as the first transversely placed ridge which encircles the restiform body in front of the clava. The way in which it turns around the restiform body

^o Loc. cit.

and ends at the attachment of the tela choroidea is particularly well shown. It compares with the right half of my figure 1 except that the free edge is apparently lacking, possibly having been torn away with the pia. Plate XXXVIII, Figs. 5 and 6, also give admirable pictures of this region; attention might be called to the free edges of the ponto-bulbar body on both sides of the former figure.

HISTOLOGICAL APPEARANCE.

On microscopical examination it is found that the ponto-bulbar body consists of a mass of multipolar ganglion cells supported in a dense neuroglia network closely resembling the nuclear masses of the pons. The lateral or free surface of this ganglionic body is entirely covered by a layer of medullated nerve fibers which in general follow the direction of the long axis of this structure and continually give off collaterals which branch among the underlying ganglion cells. On its median surface throughout its greater extent there is no such layer of fibers present and consequently the ganglion mass lies directly adherent to the adjacent structures and fuses more or less completely with them. The ponto-bulbar body presents a uniformity of histological structure throughout, so that in passing from section to section the same characteristics can be made out everywhere. The cells lie very close to one another and in large areas are separated from each other by less than the width of a cell. The narrow space between the cells is found to be filled by a dense framework of neuroglia and an intricately interwoven network of very fine nerve fibers, which in their complexity and number resemble the hypoglossal nucleus. On the other hand this rich felt-work stands out in contrast to the ala cinerea, which in Weigert sections gives such a clear picture.

The cells are of the multipolar type and show several different forms; many are spindle-shaped with their long axes parallel to the fibers; while most have the characteristic multipolar form. The former are found in greater numbers in the part of the ponto-bulbar body which lies lateral to the restiform body, the latter in the rest of the body. The size is fairly uniform, so that, of cells which were measured in sections stained in thionin, the average was $23 \times 14.5 \mu$, some cells being as large as $29 \times 17 \mu$, others only $17 \times 10 \mu$. This corresponds exactly to measurements taken of the ganglion cells scattered through the pons. To afford a means of comparison, cells from the hypoglossal nucleus were measured in the same sections and were found to be $40 \times 23 \mu$. These measurements

were made on celloidin sections of tissue which had been fixed in formalin and mordanted in a double chrome salt.

A section in any part of the ponto-bulbar body shows the free border of the nucleus outlined by a layer of nerve fibers cut either transversely as in Figs. 3, 4, 5, 6, and 7, or longitudinally as in Figs. 8, 9, 10, and 11; in no case was there a stratum of cellular material on the surface. The diameter of these fibers, including their medullary sheath, ranges from $0.8 - 2 \mu$; those making up the larger strands $1 - 2 \mu$ thick, while those between the cells generally 0.8 in thickness. This small size makes the fibers easily distinguishable from the surrounding structures with which they come into close relation. The fibers and cells together form a structure whose shape varies in section but the relation of cells to fibers are always such that masses of nuclear material lie between the fiber bundles and the rest of the brain. Occasionally only do we find strata of cells between the fiber bundles as in Figs. 9 and 10.

For the sake of simplicity it might be well in describing its finer structure to arbitrarily divide the ponto-bulbar body into a pontine part which fuses with the ventro-lateral surface of the pons, a middle part which extends median to the ventral cochlear nucleus and is perforated by the glosso-pharyngeal roots, and lastly a broad caudal part which overlaps the restiform body. With this in mind each part will be treated separately as was done in the gross description.

In the region of the trigeminal nerve small strands of fine fibers separate from the transverse fibers of the pons and turn caudally, forming rather compact small bundles which lie on the ventro-lateral surface of the pons just behind the root bundles of the fifth cranial nerve. The addition of fibers from the lateral side or middle peduncle is comparatively small, while the strands from the pons proper continue to turn in almost as far caudal as the emerging facial nerve. The nerve fibers gradually unite to form more or less rounded bundles which are spread out on the ventro-lateral border of the pons. Although there is this gradual increase of fibers from both sides, the cross section does not increase proportionately as it runs caudally. The reason for this is found to be due to rather large bundles of fibers which separate from the other fibers and run into the middle peduncle of the cerebellum.

In the region of its most cephalic part, the ganglionic mass adjacent to the fibers is very small; but as it approaches the caudal border of the pons, it increases in amount, fusing so completely with the pontine nuclei that no sharp line of demarcation can be drawn. This is well illustrated in Figs. 3 and 4, where the fibers, cut transversely, can be seen on the

surface of the pons between the facial and acoustic nerves. The nuclear mass which lies adjacent to the fibers can be traced serially back into the caudal tip of the process, but it will be noticed in these two sections that the nuclear mass is directly continuous with that scattered throughout the pons. Fig. 4 shows the *nervus intermedius* passing through the ponto-bulbar body on its way to join the facial nerve in the same manner as the glosso-pharyngeal nerve does farther caudally.

We may say its middle division begins at the point where the ponto-bulbar body leaves the pons between the facial and acoustic nerves. It lies ventral to the restiform body and all the fibers in our sections, as in Fig. 5, are cut across transversely—that is they are running caudo-cephalically. Here again it will be noted that the fibers are arranged along the surface. Throughout the length of the ventral portion numerous small strands of obliquely cut fibers are found scattered in the nuclear mass. By carefully following the sections serially it becomes evident that these small bundles are passing between the restiform body and the ponto-bulbar body. These nerve fibers leave the restiform body and travel obliquely out across the nucleus in a caudal direction and finally take their position on the surface of the nucleus, thereby swelling the number of fibers. As we pass back and forth in serial sections, a variability in the number of fibers cut transversely, calls attention to the fact that there are not only fibers arising in the nucleus itself and running along the edge, but that there are also fibers which are passing into other structures. These obliquely cut fiber bundles can be seen in Figs. 5 and 6, between the superficial fringe of transversely cut fibers and the restiform body. The exchange of fibers between the ponto-bulbar body and the restiform body takes place even in the regions where the nucleus has fused with the pontine nuclei and although the obliquely cut fibers with the same directions persist, the cross fibers from the pons and the trapezoid body make it impossible to trace bundles from the ponto-bulbar body to their junction with the restiform body. The ponto-bulbar body is separated from the restiform body by the trapezoid body as soon as the level of the ventral cochlear nucleus is reached. The separation is partial in Figs. 5 and 6, and complete in Figs. 3 and 4, where the trapezoid body can be traced to the superior olive. Attention might be called to Fig. 7, where the coarse fibers of the most cephalic of the glosso-pharyngeal nerve roots may be followed from the fasciculus solitarius through the descending spinal root of the fifth nerve and the restiform body and out through the most ventral part of the ponto-bulbar body. Fig. 7 also shows the dorsal cochlear nucleus in its relation to the ponto-bulbar body which, a

few sections farther caudally (c.p. Fig. 8), occupies a similar position with regard to the restiform body. Sections like Fig. 8 might easily be misinterpreted as containing a dorsal cochlear nucleus.

The caudal part embraces that portion of the ponto-bulbar body which sweeps around the restiform body to gain the dorsal surface of the medulla. As its most cephalic border is reached, the fibers are cut a little more obliquely indicating that they are turning out of their caudo-cephalic course, see Fig. 8 and Fig. 9. A little farther backward the fibers run around the restiform body in a rather heavy layer (Fig. 10); the nuclear mass, on the other hand, does not follow them immediately, so that in sections where the front part appears, fibers alone are seen encircling the restiform body—c.p. Fig. 8. When the body reaches its greatest development, the fibers can be traced for some distance as they bend around the restiform body; always separated from it by a mass of nuclear material. It is here that the fiber bundles which skirt along the edge may be interspersed with thin strata of ganglion cells as in Figs. 9 and 10. From the part of the nucleus which adjoins the median vestibular nucleus a few small strands of fine fibers could be traced as they ran cephalically toward one of the striæ acusticæ, which had a caudal direction. On reaching this stria, the fibers made a sharp turn and joined it. Numerous other smaller groups of fibers had a similar direction and gave the impression of joining the striæ but only one such bundle could be followed from the ponto-bulbar body to the striæ acusticæ. It is probably this connection which led Clarke to interpret this structure, which he undoubtedly saw, as belonging to the group of striæ acusticæ.

The caudal tip extends out as a tongue-like structure which projects to a greater or less extent into the roof of the fourth ventricle in the edge of the tela choroidea inferior, see Figs. 9, 10, and 11. The ventral side of the free process is lined by a layer of ependyma which is continuous with the ependymal cells lining the rest of the ventricular cavity, while the dorsal side is covered by pia mater—in every respect then it appears to be an enlarged portion of the secondary “Rautenlippe” of His. An illustration not unlike my Fig. 9 is given by Spalteholz⁷ in his figure 727. Here it appears to be disregarded as an individual structure and has no label referring to it; the characteristic superficial fiber layer, the fusion of the cell mass with the restiform body, and even the

⁷ Werner Spalteholz: *Hand Atlas of Human Anatomy*, Vol. III, Leipzig, 1906.

tongue-like process are well illustrated. His figure 728 has almost the same level as Fig. 5, and here he has called it "pons (Varoli)." This latter is very natural when we consider that a little farther front the ponto-bulbar body is actually fused with the pons.

In the tongue-like projection the cells occupy a central position, being surrounded on all sides by the fiber bundles which may then be regarded as forming a capsule; Figs. 9, 10, and 11 show this very well. Single fibers may be traced for some distance in a section showing that here in the dorsal portion the fibers take the same direction as the body that is toward the median line. These fiber strands run along the border of the nucleus and converge as they turn around to gain the lateral side of the restiform body forming a bundle which is gathered together in wisp-like fashion. The efferent fibers from the abducens, facial, and other nuclei have this same confluence into a root bundle. Toward the median end of this projection appears a rather large bundle cut transversely which gives off collaterals to the nuclear material in a very irregular manner.

The above description applies to adult material, but I have also had the opportunity of examining a series of the new-born babe, the one which was prepared by Dr. John Hewetson and belongs to the collection of this laboratory. In this series the ponto-bulbar body could be distinctly made out, and in its relations to the surrounding structures showed the same features as described in the adult. However none of its fibers are as yet myelinated, the non-myelination made it particularly easy to identify it in the region of the cochlear nerve, ventral cochlear nucleus, and trapezoid body, all of which have at this age acquired their myeline sheaths. Further caudally where it lies against the side of the restiform body it comes into contact with the cerebello-olivary fibers. Although these unmyelinated fibers fuse with the ponto-bulbar body, the closely arranged ganglion cells of the latter are easily distinguished from the coarse nerve fibers. The pons with which this body fuses cephalically also contains no myelinated fibers. It may also be added that it is present in the early stages of both human and pig embryos. It can be seen in the reconstructions of this region in a 50 mm. human embryo which are about to be published by Dr. Streeter, and it was also noted by him in dissections of pig embryos.⁸

⁸G. L. Streeter: Development of the Membranous Labyrinth and the Acoustic and Facial Nerves in the Human Embryo. Amer. Jour. of Anat., Vol. VI, p. 163.

In conclusion, I wish to acknowledge my indebtedness to Dr. George L. Streeter, at whose suggestion and under whose supervision the study of this ganglion mass was carried on. I also desire to express my thanks to Prof. Max Brödel for his assistance in preparing Figs. 2 and 12.

ABBREVIATIONS USED IN FIGS. 3-12.

- Brach. pont.* = Brachium pontis.
Corp. Rest. = Corpus restiforme.
Corp. Trap. = Corpus trapezoideum.
Fibr. Cereb. Oliv. = Fibræ cerebello-olivares.
N. Coch. = Nervus cochlearis.
N. Vest. = Nervus vestibularis.
N. Glossoph. = Nervus glossopharyngeus.
Nu. Coch. Dors. = Nucleus cochlearis dorsalis.
Nu. Coch. Vent. = Nucleus cochlearis ventralis.

EXPLANATION OF FIGS. 3-11.

FIG. 3. Transverse section through the pons immediately in front of the emerging facial nerve. $\times 4.5$.

FIG. 4. Transverse section just caudad to the preceding, through the nervus intermedius, showing the fibers of the corpus ponto-bulbare in cross section. $\times 4.5$.

FIG. 5. Transverse section through the medulla at the border of the pons. $\times 4.5$.

FIG. 6. Transverse section through the cephalic end of the olive. $\times 4.5$.

FIG. 7. Transverse section through the caudal border of the dorsal cochlear nucleus. $\times 4.5$.

FIG. 8. Transverse section through the cephalic border of the corpus ponto-bulbare where it encircles the restiform body. $\times 4.5$.

FIG. 9. Transverse section immediately caudad to the preceding, showing the longitudinally cut fiber strands turning around the restiform body. $\times 4.5$.

FIG. 10. Transverse section through the thickest part of the projecting caudal tip of the corpus ponto-bulbare. $\times 4.5$.

FIG. 11. Transverse section through the middle of the olive, showing the caudal end of the corpus ponto-bulbare. $\times 4.5$.

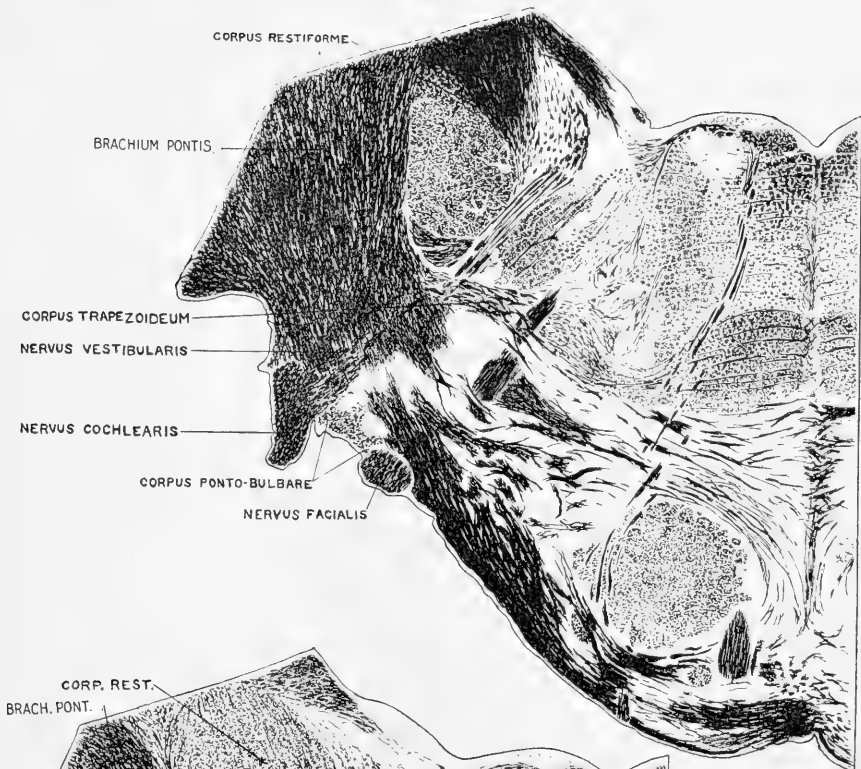


FIG. 3.

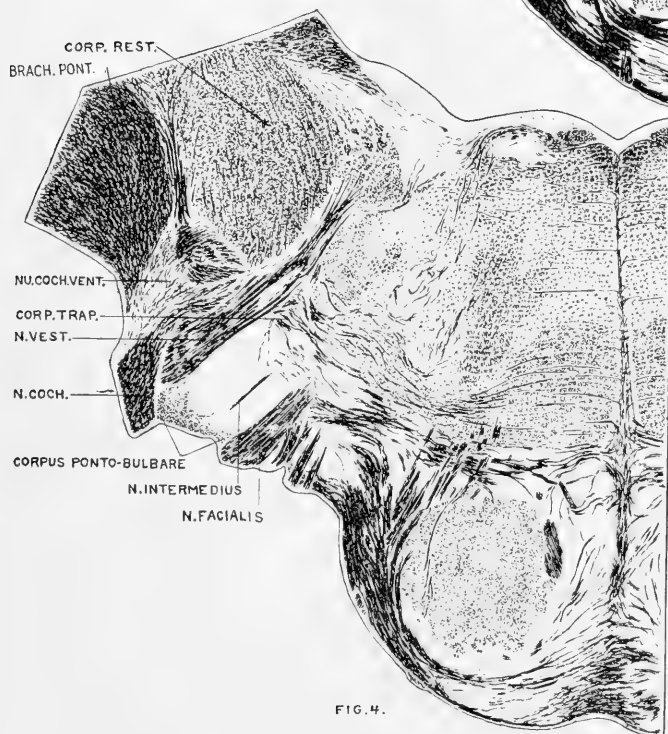
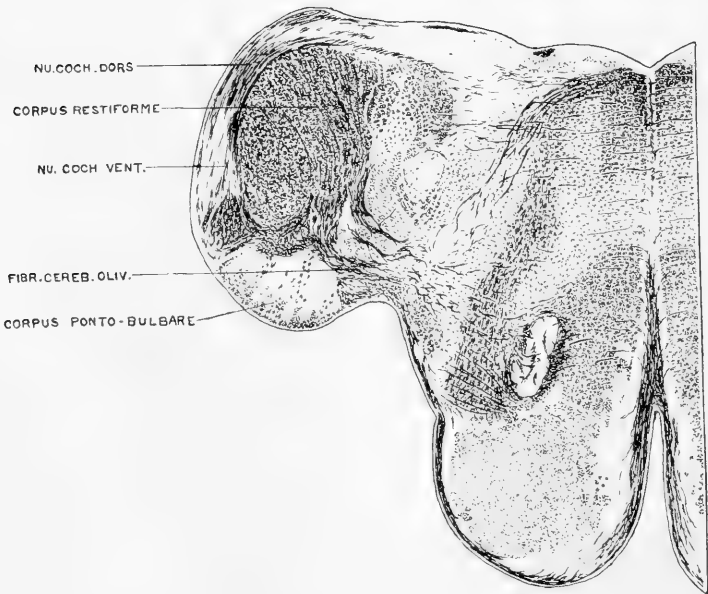
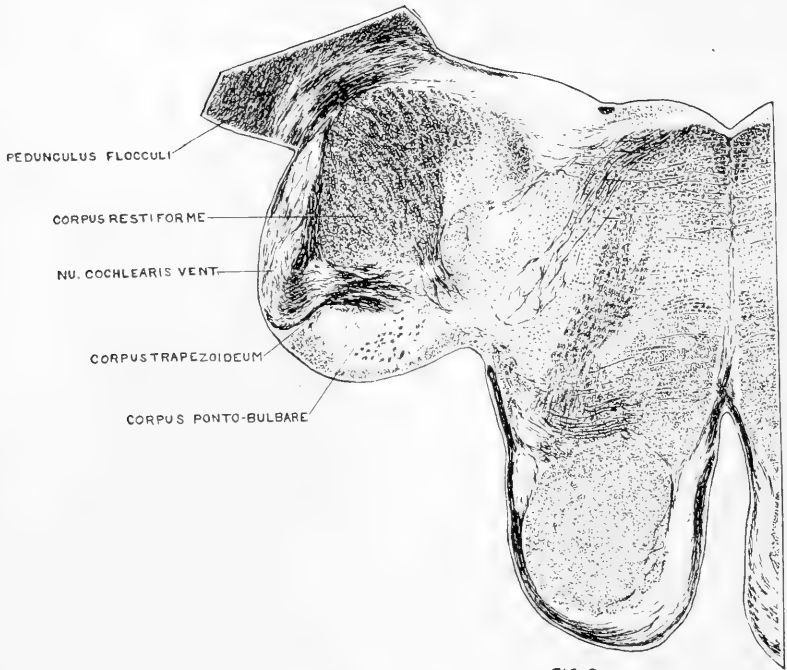


FIG. 4.



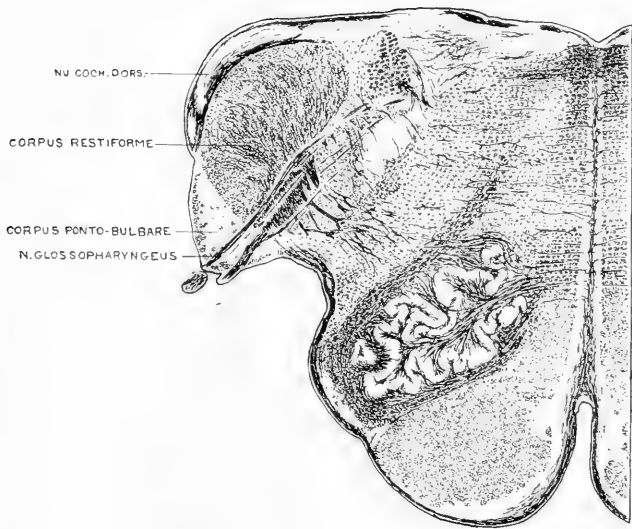


FIG. 7.

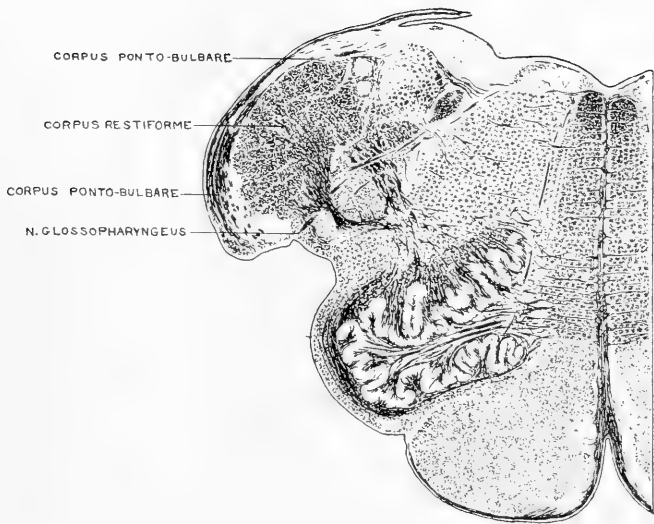


FIG. 8.

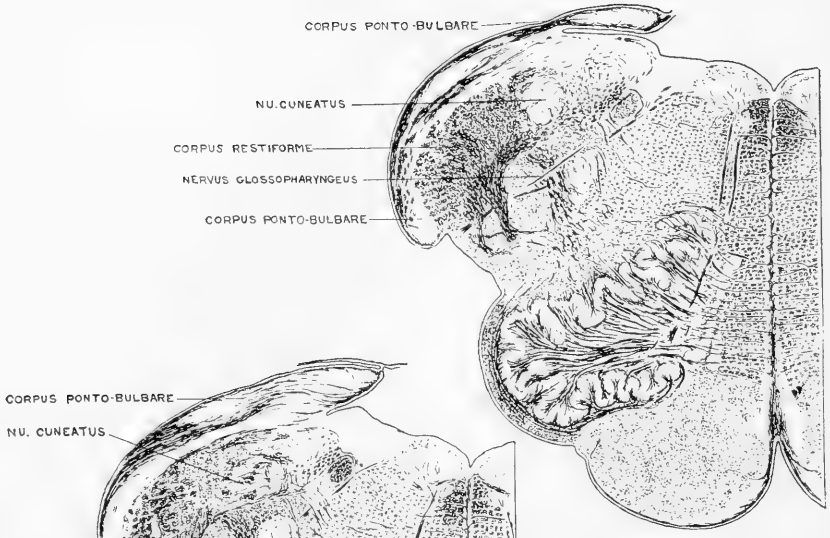


FIG. 9.

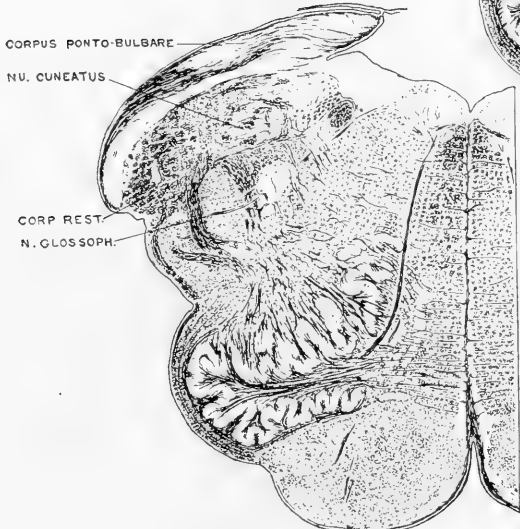


FIG. 10.

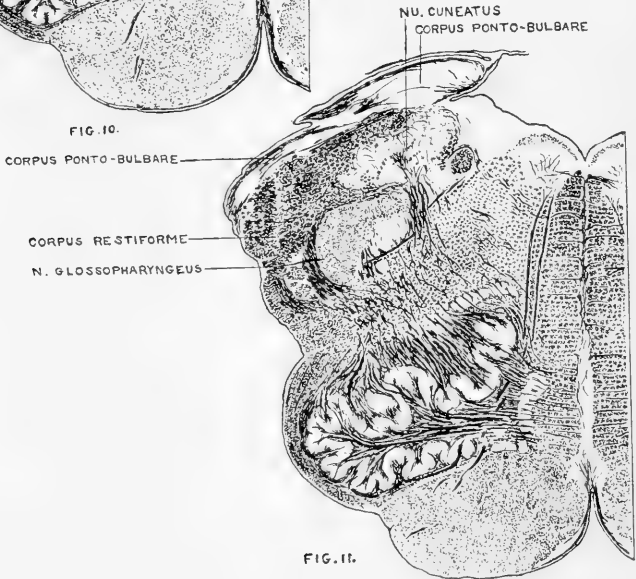


FIG. 11.

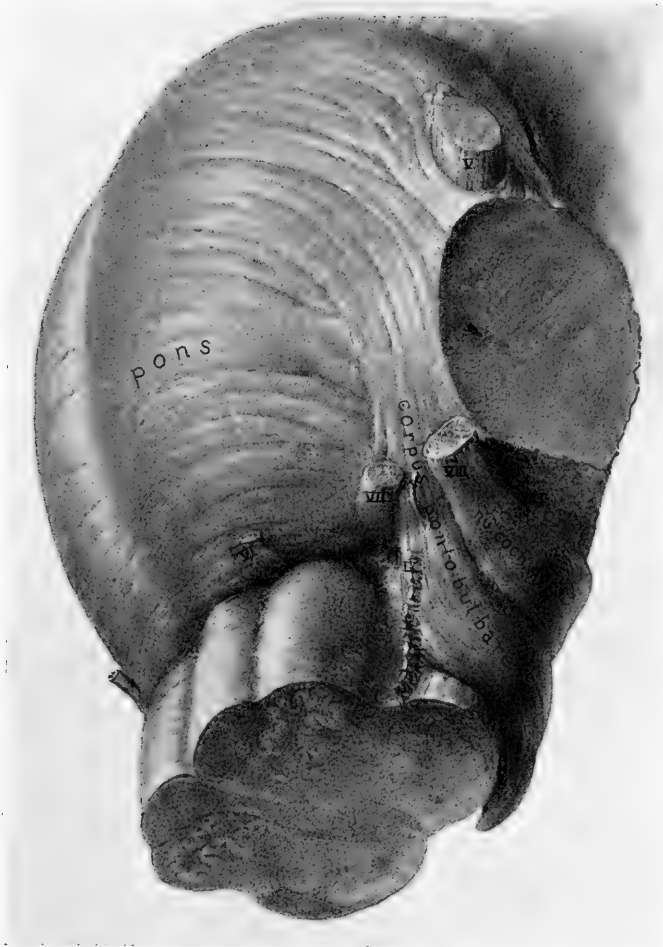


FIG. 12 represents a somewhat schematic drawing of a model reconstructed from sections of the specimen shown in Fig. 1, in a ventro-lateral view of the medulla and pons. Part of the nucleus cochlearis dorsalis (nu. coch. dors.) has been removed to show the relations of the corpus restiforme (c. r.) Numerals V-X refer to the cranial nerves. I refers to the nervus intermedius. $\times 3.75$.

TRANSPLANTATION OF THE LIPS OF THE BLASTOPORE IN
RANA PALUSTRIS.

BY

WARREN HARMON LEWIS.

From the Anatomical Laboratory, Johns Hopkins University.

WITH 5 FIGURES.

Roux¹ first pointed out that the material which forms the embryo of the frog is laid down in the black-white ring around the equator of the egg, and that the embryo is formed by a process of concurrence in that this material grows over the white hemisphere. If this is prevented, as in his case of *asyntaxia medullaris*, a half embryo develops on either side of the equator of the egg. Roux's observations and experiments were confirmed by Morgan² in a somewhat similar series of experiments. Hertwig³ was able to produce the bilateral half embryos by allowing the eggs to develop in salt solutions. I have seen many similar forms from eggs of *rana sylvatica* and *rana palustris* which were kept on ice for a prolonged period of time. In such abnormal forms the lips of the blastopore which fail to grow over the large yolk plug differentiate into these modified half embryos with a central nervous system, muscle, the chorda, and the roof of the archenteron.

More recently Morgan⁴ has investigated the question of the location of the embryo-forming substances and concludes that the material is first in the upper hemisphere of the developing frog's egg and is later carried downward into the germ ring.

¹ Ueber die Lagerung des Materials des Medullarrohes in gefurchten Froschei. *Anat. Anz.*, Vol. 3, 1888.

² The formation of the embryo of the frog. *Anat. Anz.*, Vol. 9, 1894.

³ Urmund und Spina bifida. *Arch. f. mikr. Anat.*, Vol. 39, 1892.

⁴ The relation between normal and abnormal development of the embryo of the frog, X. A re-examination of the early stages of the normal development from the point of view of the results of abnormal development. *Arch. f. Entwicklungsmech.* XIX, 1905.

The origin of the organ-forming materials in the frog's embryo. *Biol. Bul.*, XI, 1906.

The following experiments were made, partly with the view of determining what organ-forming stuffs are present in the lips of the blastopore; but more especially to determine the extent of independent self-differentiation possessed by this structure when small pieces were isolated and transferred into strange environments. In the examples of *asyn-taxia medullaris* there develops from the lips of the blastopore on either side a half embryo with spinal cord, notochord, and myotomes, so that evidently the fusion of the lips is not necessary for their differentiation, but it might be, of course, that some of the other relations are essential, such as the anterior, posterior, or the lateral connections with the embryo. But by cutting out and transplanting small sections of the lips these factors were eliminated.

The first series of experiments were made on *rana palustris* at a time when the dorsal and lateral lips are well marked, the ventral lip is just beginning to appear. Small blocks of tissue were cut from the dorsal and lateral lips (1, 2, 3, and 4, Fig. 1), so as to include the entire thickness of the lip with both ectoderm and endoderm. These pieces were transplanted beneath the ectoderm of older embryos of the same species in the region of the otic vesicle. The embryos into which they were transplanted show the beginnings of the tail bud. The pieces transplanted are soft and delicate, and of course very liable to injury during the operation by distortion, tearing, or loss of a portion.

In the first experiment (ta_1), piece (1), Fig. 1, was transplanted into an older embryo which was killed 7 days after the operation. The sections (Fig. 2) show in the region of the otic vesicle a large notochord, perfectly normal in its differentiation and extending for many sections in a direction parallel to the long axis of the central nervous system. Portions of the chorda are irregular in outline and it is not quite as large as the normal one in cross diameter. Near the chorda and ventral to the otic vesicle is a large mass of muscle, irregularly arranged, normal in its differentiation, showing striation and other characters of myotomic muscles. I am unable to find any nerves going to this muscle mass and it lies in a position where normally there is no muscle.

Ventral to the notochord and medial to the muscle mass is a piece of central nervous system having both white and gray substance and a central canal. In places the nerve cells seem to be degenerating. The central canal is closed at either end.

I am unable to determine if any of the transplanted tissue differentiated into endoderm, though some degenerating cells in this region may

possibly be endoderm. It is evident from this experiment that tissue from the dorsal lip of the blastopore possesses great power of self-differentiation, is already predetermined, and does not need the usual normal relations with the rest of the embryo for its differentiation. At how early a period these tissues are capable of independent self-differentiation is, of course, only possible to determine by further experiments along perhaps somewhat similar lines of transplantation.

The transplanted piece in another similar experiment (ta_2) of this series, taken from the region (2) (see Fig. 1), differentiated into notochord, striated muscle, and nervous tissue. The latter, however, is only imperfectly differentiated and shows many degenerating cells. This embryo was killed 7 days after the operation.

In another experiment of the same series (ta_3), piece (3) (see Fig. 1), was transplanted and the embryo killed after 7 days. There differentiated from it a large mass of striated muscle without nervous connection and a small piece of the neural tube consisting of a single layer of cells (see Fig. 3). No traces of notochord were found so it seems probable that the cells of the transplanted piece destined to form chorda were lost during the operation. The more lateral position of piece (3) does not explain its absence as from piece (4) (see Fig. 1, experiment ta_4), a large normal appearing notochord and neural tube tissue differentiated, the latter contains many degenerating cells. No muscle tissue was found and its rudiment was probably lost in transplantation. Both of the above embryos were killed 7 days after the operation.

In another series (tb) four pieces from the dorsal lip of the blastopore of a gastrula, the same age as in the preceding series, were transplanted into the otic region of similar older embryos, with beginning tail buds. These embryos were killed 6 days after the operation. As in the preceding series muscle and chorda develop into quite normal tissue, the former without any nervous connection. The neural tube is always present but shows more or less extensive degenerative changes.

In one of these experiments (tb_3) the chorda is in contact with the ectoderm, the latter shows here a modification of its staining reaction with hæmatoxylin and Congo red. Here, also, the cells of the inner layer of the ectoderm are elongated in an axis perpendicular to the surface. These modifications were very likely brought about in some way through the contact influence of the notochord.

In another similar series (te) an embryo (te_2) was killed 9 days after the operation. The sections show ventro-lateral to the otic vesicle

a well-formed chorda, a group of striated muscle fibers which seem to be spreading out in the form of a muscle (*m, m*, Fig. 5), and a small and imperfect neural tube. The other experiments in this series show that the dorsal lip has differentiated into chorda, muscle, and nervous system, but all three tissues are not always present, however, owing to loss, probably in transplantation, of their rudiments. The mutual relation of these three tissues varies in such a manner that there is evidently no interdependence, as regards differentiation. The muscle develops perfectly normally without any nervous connection for 9 days at least after the transplantation, as in none of the experiments can nerves be traced to the muscles, either from the host or the transplanted nervous tissue.

These tissues do not seem to influence the configuration or arrangement of the connective tissue about them in any especial manner nor with the exception of the one instance where the chorda has modified the ectoderm do they influence other tissues in the region in which they are developing. In one instance (*te₇*) the chorda rudiment was evidently transplanted near the normal chorda and has differentiated into a chorda, lying parallel to the normal one, both are encased in cartilage of this region, and in some places there seems to be a slight excess of cartilage about the abnormally placed chorda.

It is possible that by the transplantation of small pieces or even groups of cells from younger and younger embryos that the localization of the primary organ or tissue-forming substances can be traced back, step by step, to their more primitive locations in the egg. It may be possible, also, to determine in these early stages correlations necessary for the formation of secondary tissues or for the differentiation of these.

In almost all of these experiments the tissues which have developed from the transplanted piece are much greater in bulk, very much greater in the case of the chorda and muscle than such a piece would have produced in the same time had it remained in the normal position in the embryo from which it was taken. This is an indication of how the neighboring parts in a normal embryo must interact upon each other, regulating the size or extent of growth for each such part. It is possible that when such pieces are freed from this influence of the whole on the part, that cell division can take place more rapidly and so produce a larger piece from the same number of cells than under normal conditions.

Although at this early gastrula stage the dorsal and lateral lips of the blastopore are already determined as regards their subsequent differentiation to give rise to chorda, muscle, and nervous tissue, there is evidently considerable difference in the power of self-differentiation in that

the chorda and muscle develop into much more normal-appearing tissue than the rudiment of the nervous system. The latter, though predetermined at this time, does not seem to be able to differentiate into perfectly normal tissue after its transplantation. It is thus probably dependent on certain, as yet unknown, relations in its normal environment for its more perfect differentiation. Just what these relational factors are or for how long a period they must act is not clear, but a somewhat later stage after the first faint indications of the medullary plate appear the nervous tissue or its rudiment possesses great power of self-differentiation.

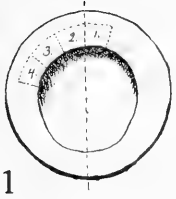
FIG. 1. Outline of blastopore region showing pieces transplanted from the dorsal and lateral lip.

FIG. 2. Experiment ta_1 . Piece of the dorsal lip of the blastopore (see (1) Fig. 1) transplanted into the otic region of an older embryo, the tail of which is beginning to show. Embryo killed 7 days after the operation. Section through otic region showing piece of nervous system (n) with central canal and marginal layer, chorda (c) and large muscle fiber mass (m). $\times 100$ diameters.

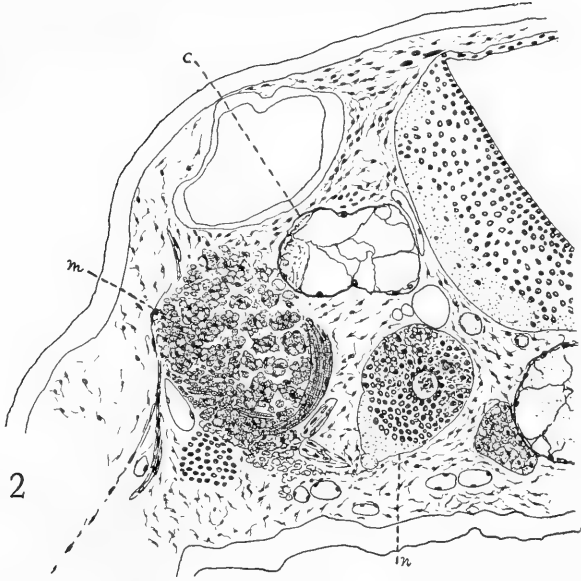
FIG. 3. Experiment ta_3 . Section through abortive neural tube, from piece (3) Fig. 1. $\times 200$ diameters.

FIG. 4. Experiment ta_4 . Section through chorda and brain from transplanted piece (4) Fig. 1. $\times 100$ diameters.

FIG. 5. Experiment te_2 . c , chorda; m, m , muscle; n , neural tube. $\times 50$ diameters.



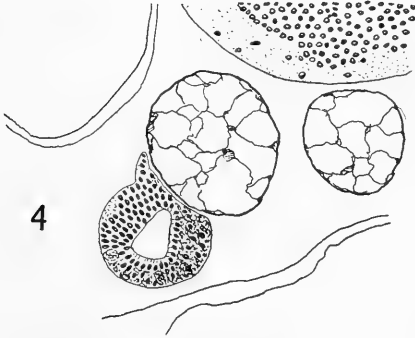
1



2



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5

LENS-FORMATION FROM STRANGE ECTODERM IN RANA SYLVATICA.

BY

WARREN HARMON LEWIS.

From the Anatomical Laboratory, Johns Hopkins University.

WITH 70 FIGURES.

That the optic vesicle can stimulate a lens to arise from ectoderm other than that which normally gives rise to one I have already demonstrated in *rana palustris*.¹ Several methods were here employed, that of transplantation of the optic vesicle into other regions of the body being the most successful. Only the earlier stages of lens-formation were shown in these experiments and it has seemed desirable to demonstrate in another species this origin of the lens from strange ectoderm and also to ascertain if such lenses from strange ectoderm are capable of development into normal lenses. *Rana sylvatica*, a common species of this region, has proved a very suitable animal for such experimentation. The right optic vesicles of 74 embryos were transplanted each into the otic region of the same side of the same embryo, from which they were removed with remarkable success as concerns the formation of lenses from the ectoderm over the region into which the eye was transplanted. I have already discussed the lens-formation in the normal region associated with regenerating eyes in this species as well as in *rana palustris*.² Here it was clearly shown that a lens will not arise from the normal lens-forming region of the ectoderm without the contact stimulus of the optic vesicle on the inner layer of the ectoderm, the lens is not a self-originating structure, nor is it a self-differentiating structure, as abortive lens-formation follows when the normal relations between lens and eye are disturbed in *rana palustris* and *rana sylvatica* and in *amblystoma*.³ It also seems, probably, from a study of the regenerating eyes that only the retinal portion of the eye is capable of stimulating lens-formation,

¹ Am. Jour. of Anat., Vol. III, p. 505.

² Am. Jour. of Anat., Vol. VI, p. 473.

³ Le Cron, Am. Jour. of Anat., Vol. VI, p. 245.

and that contact between optic vesicle and ectoderm is also necessary as the optic vesicle evidently has no power of acting at a distance to start lens-formation. Moreover, not only contact but actual adhesion between optic vesicle and ectoderm seem necessary. Such adhesion always takes place in the course of normal lens-formation. The size of the early lens structure, the lens plate, the lens bud, and the lens vesicle, is dependent in part at least upon this area of contact or adhesion between optic vesicle and ectoderm and in part also upon the length of time the optic vesicle or optic cup remains in contact by its retinal layer with the developing lens.

ANATOMY OF THE OPERATING STAGE AND METHOD OF OPERATION.

The conditions in *rana sylvatica* and *rana palustris* are essentially the same at this early stage, namely, at the time of, or shortly after, the closure of the neural folds. The anatomy of the eye region I have already described.⁴ The optic vesicle projects from the ventral one-half of the lateral surface of the brain and is in contact with the ectoderm but not adherent to it. There are, of course, no signs of lens-formation nor is the optic vesicle adherent to the ectoderm. The optic vesicle was transplanted into the otic region in each embryo (Fig. 1).

The embryos were operated upon under a binocular microscope. An incision was made caudal to the bulge formed by the optic vesicle, a skin flap turned forward from over the eye and the optic vesicle cut off with a sharp needle or a fine pair of scissors (see Fig. 3a *Am. Jour. of Anat.* Vol. VI, p. 493). From the caudal edge of the incision a pocket was made beneath the ectoderm of the otic region. In this region (Fig. 1) at this stage there is a thickening of the inner layer of the ectoderm forming the rudiment of the otic vesicle. In pushing the needle back and forth to make the pocket between ectoderm and mesenchyme the ectoderm was often injured over the pocket and also at the edge of the pocket, and even pieces of the ectoderm were detached and left in the wound and later carried into the mesenchyme with the eye. After formation of the pocket the optic vesicle was pushed into it with a blunt needle. Sometimes it slipped in quickly and without much injury, often, however, it must have been torn and distorted. The mesenchyme was always more or less injured and often some of it taken out to make room for the large optic vesicle, as one can see from Fig. 1 there is not much space between ectoderm and entoderm. In most of the embryos at the

⁴ *Am. Jour. of Anat.*, Vol. VI, p. 474, and figures 1, 2, and 3.

time of the operation one cannot tell the exact position of the transplanted eye, but sometimes a bulge of the ectoderm indicates its position. After transplantation the skin flap was turned back over the stump and held in place by turning the embryo over onto the operated-on side.

Great care was taken not to injure the normal lens region of the ectoderm. It can easily be seen also that no ectodermal cells from this region were transplanted with the eye. Healing takes place rapidly, an hour or even less suffices for closure of the wound.

The embryos were killed at varying ages in Zenker's fluid embedded in paraffin cut into serial sections 5 to 10 μ in thickness and stained in hæmatoxylin and Congo red.

RESULTS OF EXPERIMENTS.

The conclusive evidence of the ability of the transplanted eye in such experiments to stimulate lens-formation from strange ectoderm is to be found when such eyes are associated with lenses or lens-like structures that are still attached to the ectoderm of the strange region. As the operating season is short and uncertainty existed as to the length of time which must elapse after transplantation before killing the embryo in order to find this condition, many operations were made in order to catch, if possible, a few at least of the lenses still attached to the ectoderm. It will be found from a study of my specimens that 3 days after the operation is the best time to kill the embryos in order to find the lenses attached to the ectoderm.

There are, fortunately, in this series a number of such lenses or lens-buds still attached to the ectoderm of the strange region into which the eye was transplanted. These offer positive proof of the ability of the optic vesicle to stimulate lens-formation from strange ectoderm, ectoderm which under normal conditions never gives rise to a lens. Fig. 2 shows such a lens associated with an eye transplanted caudal to the otic vesicle. Its origin from the inner layer of the ectoderm is better shown in Fig. 3 and the size and shape of the lens in Fig. 4. It is very similar to the normal lens vesicle shown in Fig. 5. Fig. 6 shows another more irregular transplanted eye lying ventral to the otic vesicle. The lens-bud associated with this eye is still broadly attached to the inner layer of the ectoderm (see Fig. 7). Fig. 8 shows another large, irregular transplanted eye with a lens-bud still attached to the inner layer of the ectoderm (see Fig. 9). This lens-bud is not as large nor as far differentiated as the normal lens vesicle (compare with Fig. 10). Fig. 12 shows another large lens-bud connected with the ectoderm by a slender pedicle

which would have soon disappeared. A section through the center of this lens-bud, as seen in Fig. 11, shows quite clearly the lens-like nature of the structure, but it is not as far differentiated as the normal one (compare with Fig. 13).

Figs. 14 and 15 show another large lens-bud associated with a transplanted eye. This lens-bud has quite a long pedicle, as seen in Fig. 15. Fig. 17 shows a very normal appearing transplanted eye with a lens vesicle which has evidently just broken away from the ectoderm, as shown in Fig. 16. This transplanted eye is about the shape of the normal but the lens is not as far advanced in its differentiation (compare with Fig. 18). Fig. 19 shows another lens-bud associated with an eye transplanted ventral to the otic vesicle. The lens-bud has evidently just broken away from the ectoderm. Its size and shape are better seen in Fig. 20. It is not as far differentiated as the normal one shown in Fig. 21. Figs. 22, 23, and 24 show another lens-bud which has just separated from the inner layer of the ectoderm, probably by an artificial break. The transplanted eye lies ventro-mesial to the otic vesicle (see Fig. 23).

The embryos in the above experiments were killed from 3 to 3½ days after the transplantation of the optic vesicle and they all show unmistakable evidence of the origin of the lens from strange ectoderm. This is seen occasionally even in embryos killed 4 days after the operation, as in Fig. 25, where one end of the lens vesicle projects towards a projection on the ectoderm. This was rather of an irregular lens vesicle, as seen in Figs. 26 and 27.

The lens in some of the embryos killed 3 days after the operation may show no signs of its origin, as in Figs. 28 and 29, although the ectoderm may, as in Fig. 29. In others, as in Figs. 30 and 31, neither lens nor ectoderm show signs of the origin of the lens, though these embryos were killed only 3 days after the transplantation of the eye. In almost all of these embryos killed 4 days after transplantation of the optic vesicle the transplanted eye and its lens are separated from the ectoderm by mesenchyme, as seen in Figs. 32, 33, 35, and 37, and there is no trace of any connection between the lens and ectoderm, except, perhaps, in Fig. 35. The lenses in these experiments are not as far advanced as the normal one (compare with Fig. 36).

In the embryos killed 5 days after the transplantation (see Figs. 34, 38, 39, and 40) there is no indication of the origin of the lens, likewise, in those killed 5½ days after the operation (see Figs. 41 and 42). The transplanted eyes in the older embryos are as a rule separated by a greater thickness of mesenchyme from the ectoderm, and both the optic

cups and the lenses are further differentiated. Fig. 42 shows such a transplanted eye fully as large, if not larger, than the normal one (Fig. 43), the optic cup is as far advanced in its differentiation and the lens is as large and as far advanced in its differentiation as the normal one.

The position of these transplanted eyes varies considerably and hence the place of origin of the lens. A few of the eyes are anterior to the otic vesicle, a number are ventral, some towards the anterior end and others towards the caudal end of the otic vesicle. Many of the eyes are caudal to the otic vesicle and a few as far caudal as the Wolffian body (see Figs. 32, 37, 38, 40, and 42). So that the lenses must have arisen from numerous different places on the ectoderm. It apparently makes no difference where the lens arises in order to have fairly normal development.

The shape of the early lens varies considerably and seems to indicate that it is a very plastic structure conforming often to the peculiar shapes of the cup-cavity found in many of the transplanted eyes (see Figs. 7, 9, 11, 14, and 27). Other factors, probably, also help to modify its shape, as pressure of the pupillary margin (see Figs. 34 and 38), or pressure of surrounding structures (see Figs. 45, 47, and 48). The long processes which often connect the early lens-bud or vesicle with the ectoderm seem to be caused, as I have already explained at some length (see *Am. Jour. of Anat.*, Vol. VI, p. 486), by the same factors which tend to force the optic cup away from the ectoderm. If the mesenchyme or otic vesicle or other structure forces the eye away from the ectoderm while the lens is still attached to both, it can be seen how such processes, as shown in Figs. 15, 19, 22, and 48, are formed. The normal eye is very firmly adherent to the lens during the early stages of its development even up to the time when it is pinched off from the ectoderm, so that the lens-bud would tend to cling to the optic cup as the latter is forced away from the ectoderm by the ingrowth of mesenchyme and an elongation of the lens process would result owing to its attachment in the ectoderm.

I believe that all of the lenses associated with these transplanted eyes were formed from the overlying ectoderm through contact stimulus of the optic vesicle. The fact that in the later stages the optic cup and its lens are separated from the ectoderm by mesenchyme is no proof whatever that it was not at one time in contact with the ectoderm and for a sufficient length of time to stimulate lens-formation. The part which the mesenchyme plays in the separation of the eye from the ectoderm is probably a very active one, and even the normal eye after the lens has separated from the ectoderm becomes separated by a layer of mesenchyme from the skin. The mesenchyme may, perhaps, be looked upon as an active in-

vader constantly struggling to penetrate between and within the various organs of the body, and so pushing them apart in one way or another. In diseased conditions, as of the liver or kidney, the resistance against its invasion is lowered, the balance lost, and as a result a connective tissue invasion occurs. In the gradual separation of the otic vesicle from the ectoderm we have an example of this action of the mesenchyme. The growth of the otic vesicle is perhaps a factor in some cases, for at the time of transplantation it consists of merely a thickening of the ectoderm (see Fig. 1), its great increase in size, as in Figs. 44, 45, and 47, may have helped some in forcing the transplanted eyes away from the ectoderm.

The fact that in the younger embryos the eyes are in contact or close to the ectoderm and that in the older embryos there is a progressive increase in the thickness of the mesenchyme would seem to speak in favor of a contact between ectoderm and optic vesicle at an early stage. Again, there are in this series a number of experiments in which the attempt was made to transplant the optic vesicle some distance beneath the ectoderm and in most of these the eye apparently remained deeply seated and without a lens, the eye not being able to stimulate at a distance lens-formation from the ectoderm.

There is, also, no indication in these early stages of lenses arising from the edge of the optic cup and the fact that the lens is often nearly as large, or as large, as normal and as far advanced or nearly so would seem to exclude this mode of origin, especially as in the early stages of the transplanted optic vesicle there is no distinct papillary margin from which the lens in older amphibian embryos has been found arise.

During the operation of transplanting the eye, the ectoderm in the region of the transplanted eye, or at the edge of the pocket, was often injured in such a way as to often bring pieces of ectoderm into the region of the transplanted eye. Such pieces of ectoderm vary much in size and shape, they may or may not remain attached to the ectoderm. If the optic vesicle comes into contact with such pieces of ectoderm a lens may arise from that portion of it in contact with the retinal layer. Such long ectodermal processes may remain attached in the ectoderm and a lens form from only a portion of it, as in Figs. 49, 50, and 51. The lens in Fig. 51 is connected on one side with a long process to the ectoderm and on the other with two irregular processes which dangle in the cavity of the optic cup. The lens evidently only formed from a small part of this process.

The lens shown in Fig. 45 may have arisen from the ectodermal

strand in its neighborhood. Some of these ectodermal masses are quite irregular and branched, as in Figs. 52, 53, 54, 55, 56, 58, 60, and 62, the lens forming from only one portion of it. Such ectodermal masses may be attached to the ectoderm or pharynx or both. Sometimes the ectodermal process or mass is free and the larger part of it developing into a lens under the influence of the optic cup, as in Figs. 61, 62, 63, 64, 66, 67, 68, and 69. The lenses forming from such masses often show retarded differentiation (compare Figs. 63 and 65).

Very often there is between the ectoderm and the pharynx wall an ectodermal connection, and the lens may arise from it, as in Fig. 70. Although these processes may be attached to the pharynx wall, as in Figs. 53, 54, 55, 58, 60, and 70, one can distinguish between the two tissues. There are a number of deeply transplanted eyes in contact with the pharynx and peritoneum, but these tissues do not seem to be able to give rise to the lens. The formation of a lens from ectoderm in the region of the otic vesicle has apparently no influence on the formation of another lens from the normal region of the same side of the head by the regenerating eye as occasionally both transplanted and regenerating eyes may show lens-formation from the ectoderm. This would seem to indicate that lens-formation is a purely local process. Nor is any connection of the transplanted eye with the brain necessary for lens-formation, as in most cases no connection whatever exists between transplanted eye and brain.

There is apparently no predetermined area of the ectoderm which must be stimulated in order that a lens may arise. As to whether there exists any polarity as regards the ease with which the ectoderm responds to the lens-forming stimulus can scarcely be determined from these experiments, as a much more complete series with eyes transplanted into various other regions would be necessary and even then it would be a difficult matter to determine if the normal lens-forming ectoderm was more readily acted upon than ectoderm farther caudal, as the difficulty of bringing the transplanted eye into as favorable relations with the ectoderm as in the normal region must be taken into consideration.

As to whether the lens can arise from any other structure as the otic vesicle, the pharynx wall, the peritoneum, the brain, etc., through the influence of the eye is in no way indicated by my experiments and there are examples where the eyes have come into contact with these structures without any signs of lens-formation.

For how long a period such transplanted eyes and lenses would continue their normal development can only be determined by further experimentation.

FIG. 1. Section through otic region of the operating stage, into which region the optic vesicle was transplanted. The rudiment of the otic vesicle is represented by a thickening of the inner layer of the ectoderm. Beneath it lies mesoderm and then the thick wall of the pharynx. $\times 100$ diameters.

FIG. 2. Experiment DL₁₄. Optic vesicle transplanted caudal to the otic vesicle. Embryo killed 3 days after the operation. Section through the region of the transplanted eye showing medulla, chorda, myotomes, etc. Associated with the transplanted eye is a large lens-bud still attached to the inner layer of the ectoderm. It is 110 μ in diameter and is as large as the normal lens but the latter has just separated from the ectoderm. The transplanted eye is as large as the normal eye but somewhat irregular in shape. $\times 45$ diameters.

FIG. 3. Experiment DL₁₄. Section through edge of the above transplanted eye and lens-bud showing the attachment of the latter to the inner layer of the ectoderm. Compare with normal lens, Fig. 5. $\times 180$ diameters.

FIG. 4. Experiment DL₁₄. Section through about the center of the lens-bud of the transplanted eye showing small cavity and elongation of the cells in contact with retina. $\times 180$ diameters.

FIG. 5. Experiment DL₁₄. Section through center of normal lens. $\times 180$ diameters.

FIG. 6. Experiment DL₁₂. Optic vesicle transplanted ventral to the otic vesicle. Embryo killed 3 days after the operation. Section through transplanted eye and lens-bud, otic capsule (*oc*), medulla, and anterior myotomes. $\times 45$ diameters.

FIG. 7. Experiment DL₁₂. Section through edge of transplanted eye and lens-bud showing its broad attachment and peculiar shape. The neighboring sections show a small cavity in the lens. The lens vesicle on the normal side is similar to that shown in Fig. 5. $\times 180$ diameters.

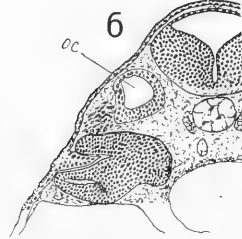
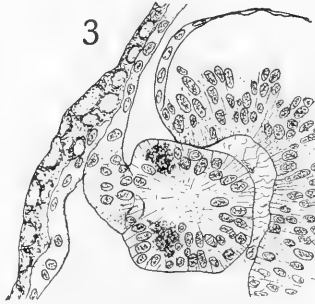
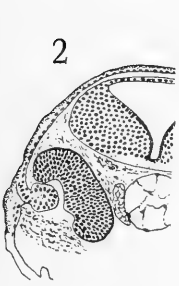
FIG. 8. Experiment DL₁₆. Optic vesicle transplanted ventral to otic vesicle. Embryo killed 3½ days after the operation. Section through anterior portion of irregular transplanted eye and lens showing medulla and ganglionic masses. This section is just anterior to the otic vesicle but the greater part of the transplanted eye lies ventral to it. $\times 45$ diameters.

FIG. 9. Experiment DL₁₆. Section through edge of above transplanted eye and lens-bud. The lens-bud is attached to the ectoderm and has a small irregular cavity. The lens-bud is much flattened and smaller than the normal one (see Fig. 10). $\times 180$ diameters.

FIG. 10. Experiment DL₁₆. Section through normal lens vesicle showing cavity filled with mass of degenerating cells. $\times 180$ diameters.

FIG. 11. Experiment DL₃. Optic vesicle transplanted ventral to the otic vesicle. Embryo killed 3 days after the operation. Section through edge of the transplanted eye and lens showing attachment to the ectoderm. $\times 180$ diameters.

FIG. 12. Experiment DL₃. Section through same eye and lens a few sections caudal to the above, showing irregular lens vesicle corresponding in shape to the eye cavity. This lens-bud is about the same size as the normal one, but the latter has separated from the ectoderm and is further differentiated (Fig. 13). $\times 200$ diameters.



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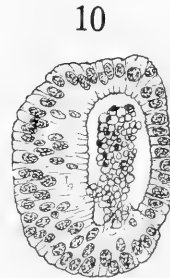
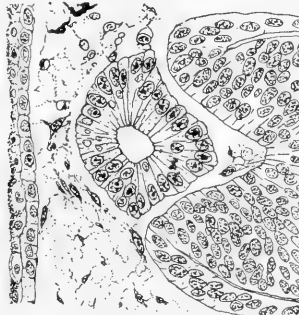


FIG. 13. Experiment DL₃. Section through normal lens. $\times 200$ diameters.

FIG. 14. Experiment DL₂₀. Embryo killed 4 days after the operation. Section through irregular transplanted eye and lens. $\times 90$ diameters.

FIG. 15. Experiment DL₂₀. Section showing the attachment of the above lens to the ectoderm. $\times 180$ diameters.

FIG. 16. Experiment DL₁₃. Optic vesicle transplanted caudo-ventral to the otic vesicle. Embryo killed $3\frac{1}{2}$ days after the operation. Section through the transplanted eye and lens caudal to the otic vesicle. The lens has evidently just broken away from its attachment to the ectoderm. The optic cup is about the same size, shape, and stage of differentiation as the normal. Compare with Fig. 18. $\times 90$ diameters.

FIG. 17. Experiment DL₁₃. Section through optic cup and center of the lens caudal to the above. The lens is not as far differentiated as the normal one but its volume is slightly greater. $\times 90$ diameters.

FIG. 18. Experiment DL₁₃. Section through normal eye and lens. $\times 90$ diameters.

FIG. 19. Experiment DL₁₀. Optic vesicle transplanted ventral to the otic vesicle. Embryo killed 3 days after the operation. Section through irregular transplanted eye and caudal end of the lens, otic vesicle, and medulla. The lens has evidently just separated from the ectoderm, very likely during the preparation of the specimen. $\times 90$ diameters.

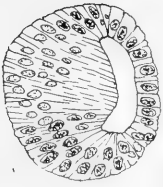
FIG. 20. Experiment DL₁₀. Section caudal to the above through edge of eye and lens. The lens is not of as great cross diameter as in the normal one, but is $\frac{1}{3}$ longer and of greater volume. The transplanted lens is not as far differentiated as the normal. Compare with Fig. 21. $\times 180$ diameters.

FIG. 21. Experiment DL₁₀. Section through normal eye and lens. $\times 90$ diameters.

FIG. 22. Experiment DL₅. Optic vesicle ventral to the otic capsule. Embryo killed 3 days after the operation. Section anterior to the otic vesicle through anterior end of eye and lens, showing the connection of the latter with the inner layer of the ectoderm. The lens is about the same diameter as the normal but is oval in shape and twice as long as wide. $\times 90$ diameters.

FIG. 23. Experiment DL₅. Section through center of lens and transplanted eye. Here the eye and lens are separated from the ectoderm by mesenchyme and the otic vesicle. $\times 90$ diameters.

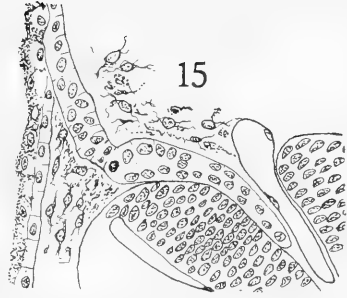
FIG. 24. Experiment DL₅. Section through caudal end of lens and edge of eye. Over the eye is a loop of ectoderm following the course of the dotted line and continuous at either end with the inner layer of the ectoderm. In this section the middle position of the loop is shown in diagonal sections. $\times 180$ diameters.



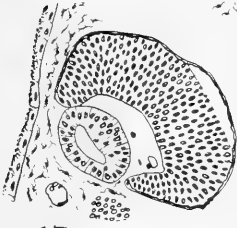
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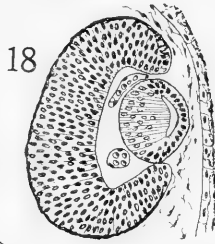
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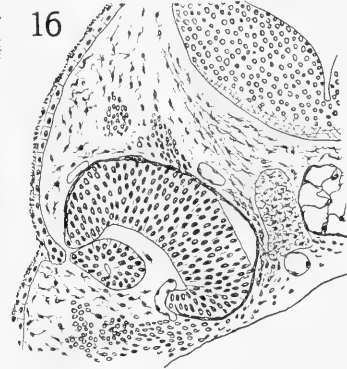
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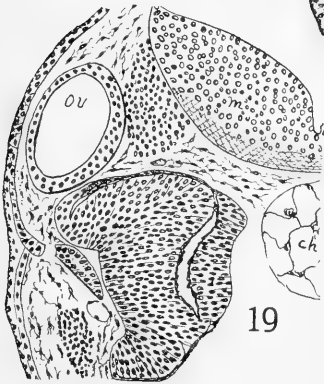
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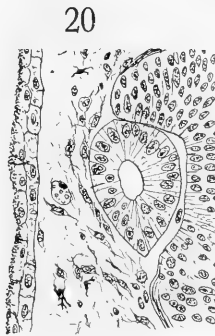
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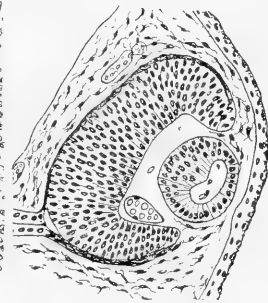
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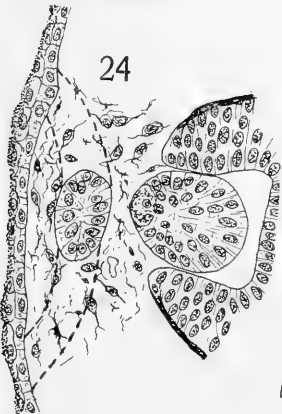
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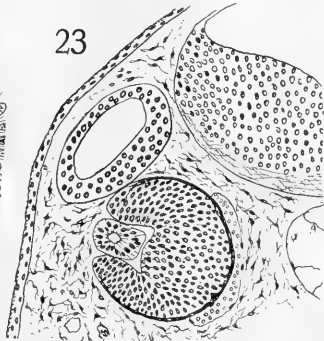
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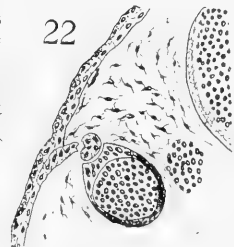
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FIG. 25. Experiment DL₁₀. Optic vesicle transplanted ventral to the otic vesicle. Embryo killed 4 days after the operation. Section through transplanted eye and anterior end of lens showing where it was attached to the ectoderm. $\times 90$ diameters.

FIG. 26. Experiment DL₁₀. Section through transplanted eye, lens vesicle, and otic vesicle, showing how the latter and the mesenchyme have pushed the eye away from the ectoderm. $\times 90$ diameters.

FIG. 27. Experiment DL₁₀. Diagram of shape of lens in a plane at right angles to the sections. The shape of the lens corresponds to that of the optic cup cavity. $\times 90$ diameters.

FIG. 28. Experiment DL₁₁. Optic vesicle transplanted caudo-ventral to the otic vesicle. Embryo killed 3 days after the operation. Section caudal to the otic capsule through irregular transplanted eye and lens vesicle. The lens vesicle is as large as the normal lens but not as far advanced in its differentiation. Compare with Fig. 13, which is similar to the normal lens in this experiment. $\times 90$ diameters.

FIG. 29. Experiment DL₁₁. Section through edge of transplanted eye, anterior end of lens and ectoderm. The latter shows place of origin of the lens, which probably has only recently separated from the ectoderm. $\times 180$ diameters.

FIG. 30. Experiment DL₆. Optic vesicle transplanted caudal to the otic vesicle. Embryo killed 3 days after the operation. Section through irregular transplanted eye and lens vesicle, and medulla. The lens a little smaller than normal is close against the ectoderm but not attached to it. The normal lens is similar to the one in Fig. 21. $\times 90$ diameters.

FIG. 31. Experiment DL₆. Optic vesicle transplanted caudal to otic vesicle. Embryo killed 3 days after the operation. Section through irregular transplanted eye, lens, and medulla. The lens is slightly larger than normal but not as far advanced in its differentiation. It is entirely separate from the ectoderm. Normal lens as in Fig. 21. $\times 90$ diameters.

FIG. 32. Experiment DL₄₃. Optic vesicle transplanted caudal to the otic vesicle and dorsal to the anterior end of the Wolffian body. Embryo killed 4 days after the operation. Section through transplanted eye and lens, medulla and anterior end of Wolffian body. The transplanted eye is of the same size, shape, and stage of differentiation as the normal one and the lens is also slightly larger but of the same stage of differentiation as the normal lens. Compare with Fig. 18, which is like the normal lens. $\times 90$ diameters.

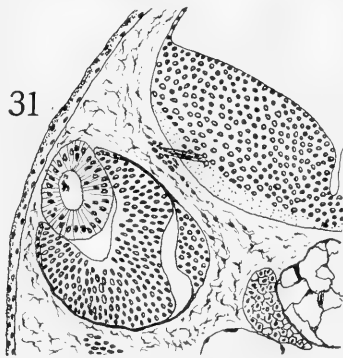
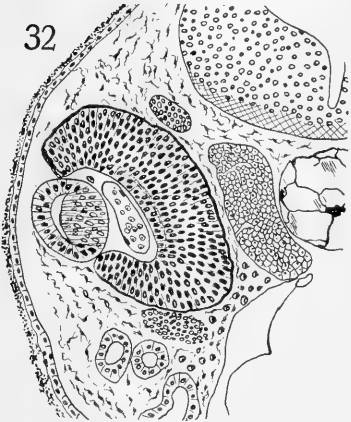
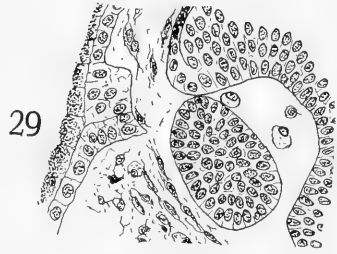
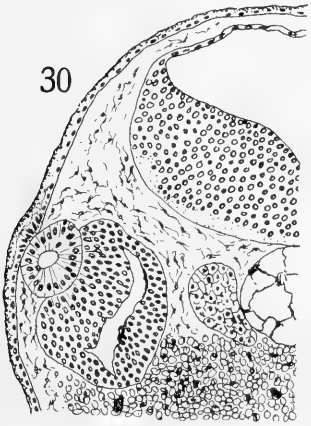
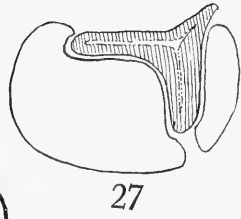
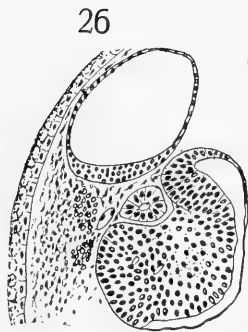
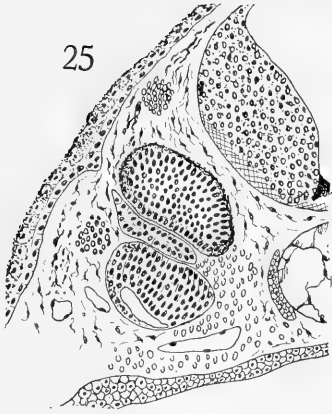


FIG. 33. Experiment DL₂₇. Optic vesicle transplanted caudal to the otic vesicle. Embryo killed 4 days after the operation. Section through transplanted eye and lens, and medulla. Both transplanted eye and its lens are larger than the normal ones but not quite as far differentiated. The transplanted eye is somewhat irregular. The medulla was injured. Compare with Fig. 36, which is similar to the normal eye of this embryo. $\times 90$ diameters.

FIG. 34. Experiment DL₂₀. Optic vesicle transplanted caudal to the otic vesicle region. Embryo killed 5 days after the operation. Section through transplanted eye, lens, and medulla. The optic cup shows regular invagination and is about the same size as normal, but not as far advanced in its differentiation. The lens is irregular in shape, as large as normal, but not as far differentiated. Compare with Fig. 36. The ectoderm over the transplanted eye shows early corneal changes. $\times 90$ diameters.

FIG. 35. Experiment DL₂₆. Optic vesicle transplanted caudal to otic vesicle. Embryo killed 4 days after the operation. Section through transplanted eye and lens. They are separated from the ectoderm by a considerable layer of mesenchyme, but the arrangement of the ectoderm and mesenchyme immediately over the lens suggest that the eye has been pushed away from the ectoderm. There is a large ganglionic mass between the eye and the ectoderm. The transplanted eye is quite irregular and has a large blood-vessel in its cavity. The lens is as large as normal but neither are so far differentiated as in the normal eye. Compare Fig. 36. $\times 90$ diameters.

FIG. 36. Experiment DL₁₇. Section through normal eye and lens. $\times 90$ diameters.

FIG. 37. Experiment DL₁₇. Optic vesicle transplanted caudal to the otic vesicle. Embryo killed 4 days after the operation. Section through transplanted eye and lens. The eye lies dorsal to the anterior end of the Wolffian body. The transplanted eye is as large and as well differentiated as normal and its lens is as large but not as far differentiated as the normal lens. A large blood-vessel occupies part of the cup cavity. The section passes through the region of the choroidal fissure. $\times 90$ diameters.

FIG. 38. Experiment DL₃₄. Optic vesicle transplanted caudal to the otic vesicle into the region of the anterior end of the Wolffian body showing tubules. The transplanted eye and lens are as large as normal but neither are so far differentiated and both are more or less irregular. Compare with normal eye, Fig. 36. $\times 90$ diameters.

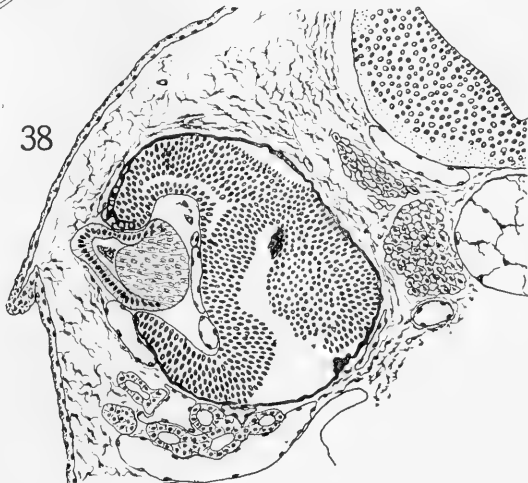
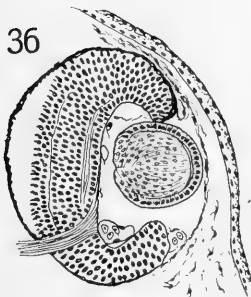
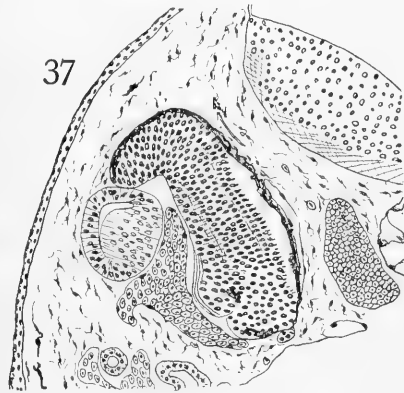
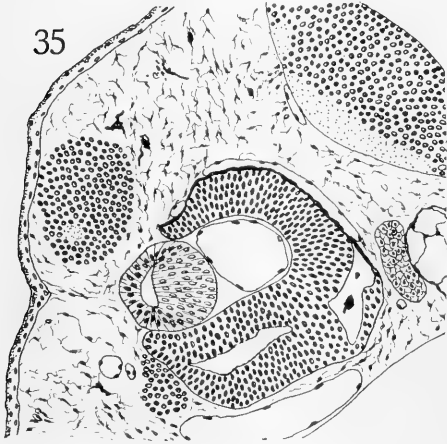
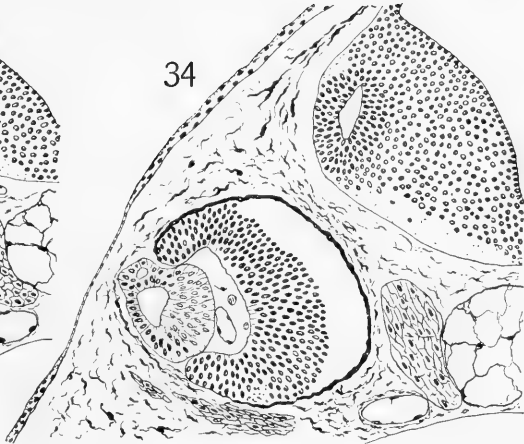
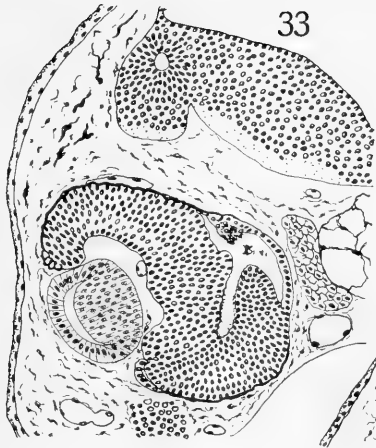


FIG. 39. Experiment DL₃₁. Optic vesicle transplanted caudal to the otic capsule. Embryo killed 5 days after the operation. Section through transplanted eye, lens, and medulla. The transplanted eye is of same stage of differentiation as normal and of about the same size or larger. The lens is nearly as large as normal but not as far differentiated. The anterior endothelial layer is forming over the lens but is not as well differentiated as normal. $\times 90$ diameters.

FIG. 40. Experiment DL₃₀. Optic vesicle transplanted caudal to the otic vesicle and dorsal to the anterior end of the Wolffian body. Embryo killed 5 days after the operation. Section through transplanted eye and lens, medulla, and anterior end of Wolffian body. The transplanted eye is fairly regular and somewhat larger than the normal eye, and as far advanced in its differentiation. Its lens is larger than the normal but not as far advanced in differentiation. The eye and lens are separated by a considerable thickness of mesenchyme from the ectoderm. $\times 90$ diameters.

FIG. 41. Experiment DL₃₃. Optic vesicle transplanted caudal to the otic vesicle. Embryo killed $5\frac{1}{2}$ days after the operation. Section through transplanted eye and lens. The transplanted eye and lens are larger than normal and as far advanced in differentiation. Compare with Fig. 43. The transplanted eye is quite irregular on one side but the portion surrounding the lens is fairly regular. The anterior endothelial layer is forming but is not as far advanced as in the normal eye. The ectoderm over the eye has a long injury process, the base of which is cut through in this section. $\times 100$ diameters.

FIG. 42. Experiment DL₃₂. Optic vesicle transplanted caudal to the otic vesicle near the anterior end of the Wolffian body. Embryo killed $5\frac{1}{2}$ days after the operation. Section through transplanted eye and lens. Both are as large and as well differentiated as normal (Fig. 43). The transplanted eye is separated by a considerable layer of mesenchyme from the ectoderm. The anterior endothelial layer is forming over the pupil. $\times 200$ diameters.

FIG. 43. Experiment DL₃₅. Section through normal eye and lens. $\times 100$ diameters.

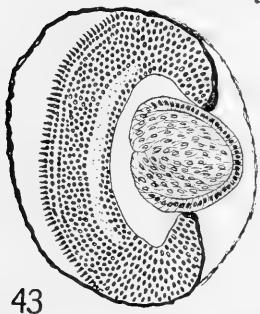
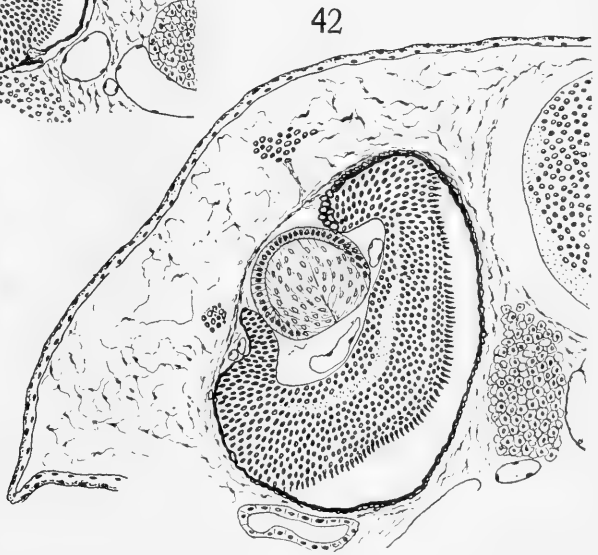
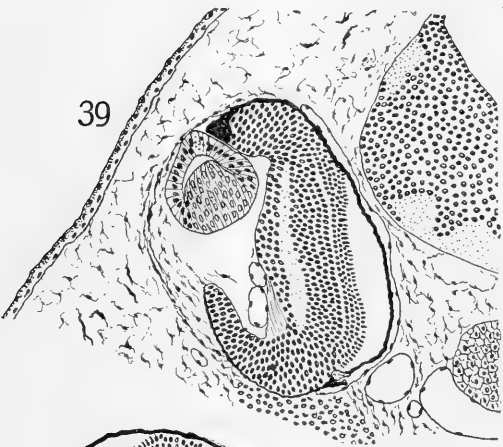
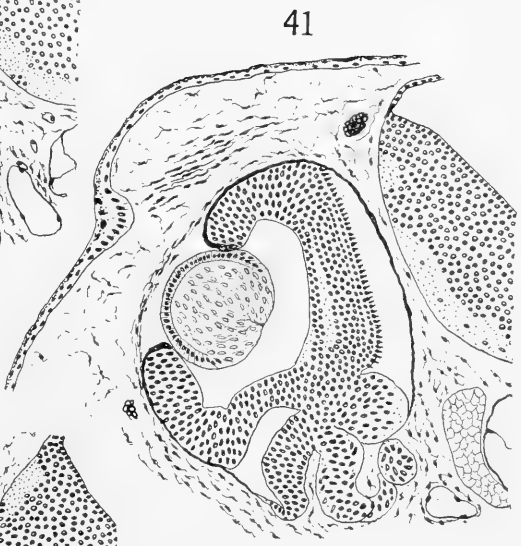
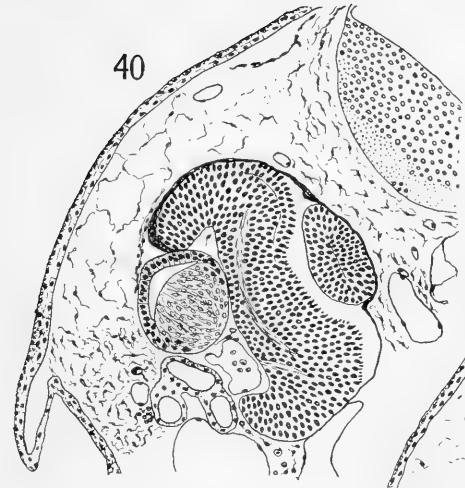


FIG. 44. Experiment DL₄₇. Optic vesicle transplanted in the region of the otic vesicle. Embryo killed 5 days after the operation. Section through transplanted optic vesicle, otic vesicle, and medulla. The main part of the optic vesicle lies medial to the otic vesicle but the anterior end of the eye lies anterior to the otic vesicle and the eye and lens here are separated from the ectoderm by a thick layer of mesenchyme. Both lens and transplanted eye seem to be larger than normal, the optic cup is somewhat irregular but as far differentiated as normal; the lens, however, is not as far advanced. Compare with Fig. 43, which is similar to the normal eye. $\times 90$ diameters.

FIG. 45. Experiment DL₄₆. Optic vesicle transplanted into otic region. Embryo killed 3 days after the operation. Section through transplanted eye, lens, otic vesicle, etc. Ventral to the transplanted eye is a large mass of modified ectodermal tissue, a process from this at (a) is connected with a long strand of ectodermal cells which pass anteriorly between the otic vesicle and optic cup close to the lens, to the ectoderm anterior to the otic capsule (see Fig. 46). The ectodermal mass is also attached to the ectoderm in the region (b) a few sections caudal to this one. Whether the lens has arisen from this long strand which lies near it or directly from the ectoderm is impossible to decide. The lens is much elongated with cavities at each end. $\times 100$ diameters.

FIG. 46. Experiment DL₄₆. Section through origin of ectodermal strand showing its continuity with the inner layer of the ectoderm. $\times 200$ diameters.

FIG. 47. Experiment DL₄₅. Optic vesicle transplanted into the region of the otic vesicle. Embryo killed 4 days after the operation. Section through transplanted eye and lens. They are separated from the ectoderm by mesenchyme and the otic vesicle. The lens is somewhat irregular and is still attached to the ectoderm by a narrow pedicle (see Fig. 48). $\times 90$ diameters.

FIG. 48. Experiment DL₄₅. Flat reconstruction of the above lens made from several adjoining sections. It shows the narrow pedicle connecting the lens with the ectoderm and also a portion of the lens between one edge of the optic cup and the otic vesicle. $\times 90$ diameters.

FIG. 49. Experiment DL₄₈. Optic vesicle transplanted anterior to the otic vesicle. Embryo killed 4 days after the operation. Section through transplanted eye and small lens-bud. The latter is attached to a large irregular ectodermal mass which has come from the inner layer of the ectoderm, probably caused by injury to the ectoderm during the transplantation. $\times 90$ diameters.

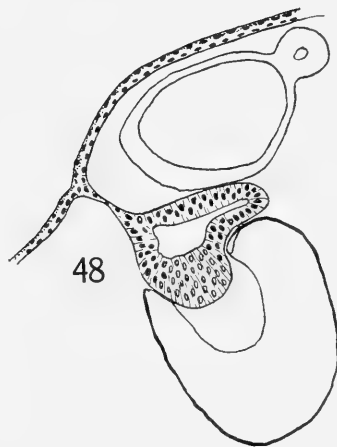
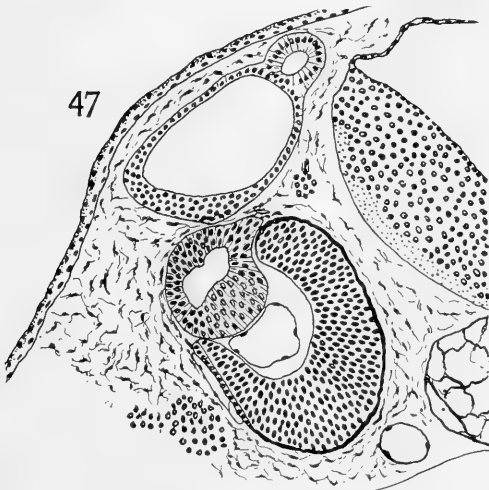
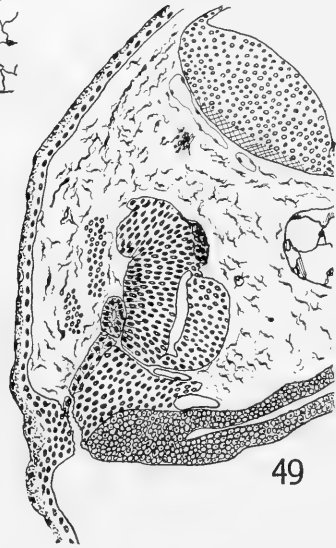
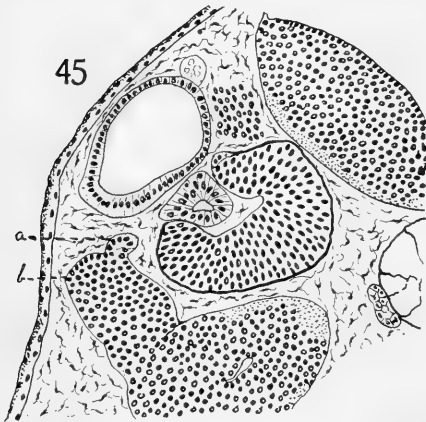
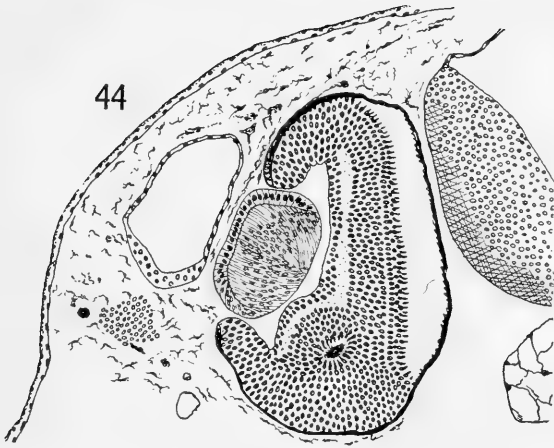


FIG. 50. Experiment DL₇₂. Optic vesicle transplanted caudal to the otic vesicle. Embryo killed 5 days after the operation. Section caudal to otic vesicle through transplanted eye, lens, medulla, etc. The pupil is directed ventrally. The lens is smaller than normal and not so far advanced. The lens is attached to the ectoderm just caudal to the eye by a long ectodermal process (see Fig. 51). $\times 90$ diameters.

FIG. 51. Experiment DL₇₂. Diagram of the lens and ectodermal process from several sections. The caudal end of the lens is attached to the ectoderm by this long process. From the cephalic end of the lens projections of ectodermal tissue extend into the cup cavity so that the lens is developing from only a small portion of the ectodermal process. Adjoining the attachment of the lens process are other irregular ectodermal projections due to injury of the ectoderm. $\times 90$ diameters.

FIG. 52. Experiment DL₂₇. Optic vesicle transplanted ventral to the otic vesicle. Embryo killed 5 days after the operation. Section through transplanted eye, lens, etc., showing deeply-placed eye. The irregular lens is connected by complicated strands of ectodermal tissue with the ectoderm and pharynx (see diagram). $\times 40$ diameters.

FIG. 53. Experiment DL₂₇. Diagram of ectodermal strands connecting lens with ectoderm and wall of the pharynx showing how the various parts, *a*, *b*, *c*, and *p*, of Fig. 52 are connected together. *e*, *e*, ectoderm; *ph*, pharynx wall. $\times 90$ diameters.

FIG. 54. Experiment DL₃₁. Optic vesicle transplanted ventral to otic vesicle. Embryo killed 5 days after the operation. Diagram of irregular ectodermal mass connected with the ectoderm and pharynx. One portion of it in contact with the eye has a large lens vesicle. *ov*, eye; *e*, ectoderm; *l*, lens vesicle; *ph*, pharynx wall. $\times 90$ diameters.

FIG. 55. Experiment DL₄₈. Embryo killed 5 days after the operation. Section through transplanted eye, etc., showing lens attached to ectodermal process which is attached to the wall of the pharynx. *oc*, otic vesicle. $\times 40$ diameters.

FIG. 56. Experiment DL₄₈. Section through irregular lens and transplanted eye. A few sections from above. $\times 90$ diameters.

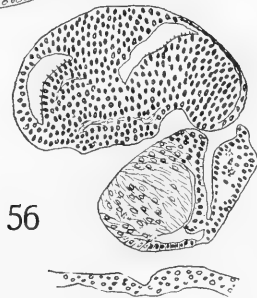
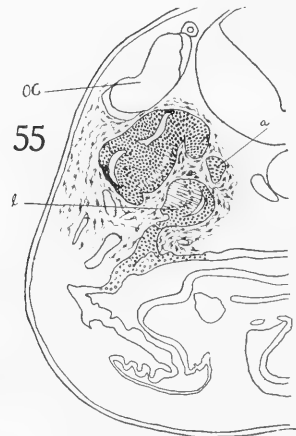
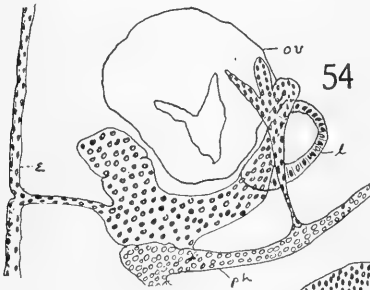
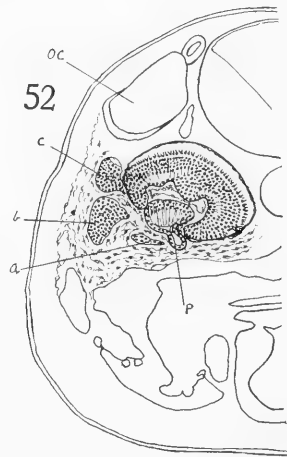
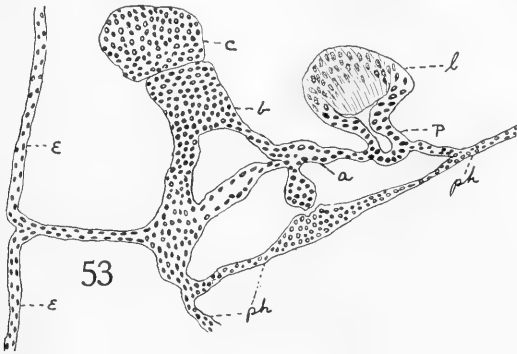
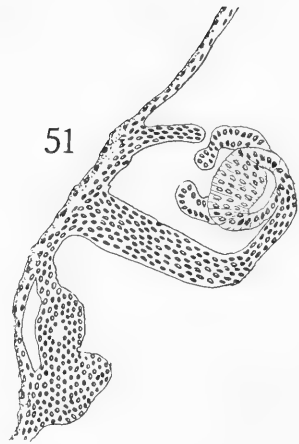
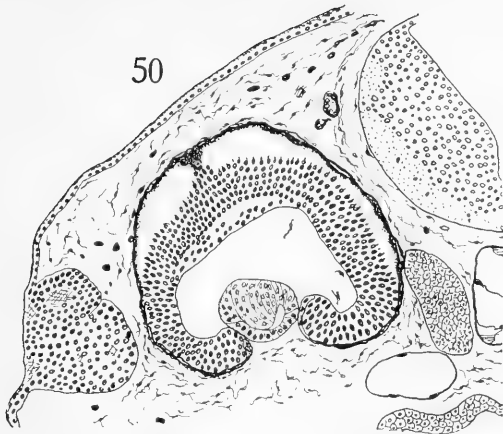


FIG. 57. Experiment DL₂₆. Optic vesicle transplanted medial to otic vesicle. Embryo killed 5 days after the operation. Section through transplanted eye and lens, medulla, otic vesicle, etc. The transplanted eye is very irregular and deeply situated. Its lens is also very irregular (see Fig. 53). The sections are not cut through the principle axis of the lens. There are two groups of lens fibers, the smaller one is in the epithelial layer. The pole of the larger group which is normally towards the retina has become shifted around and appears to be directed towards the lens cavity. $\times 90$ diameters.

FIG. 58. Experiment DL₂₀. Diagram of irregular lens with its ectodermal processes made by projection of several adjoining sections. One part projects between otic vesicle (*oc*) and the edge of the optic cup. On the other side of the lens a large irregular ectodermal process runs to the roof of the pharynx and is fused in several places with it. The line of fusion is readily distinguished. $\times 100$ diameters.

FIG. 59. Experiment DL₄₉. Embryo killed 5 days after the operation. Section through irregular transplanted eye and lens. The irregular lens is connected in two places with an irregular branching mass of ectoderm which in turn joins the roof of the pharynx. $\times 90$ diameters.

FIG. 60. Experiment DL₁₀. Diagram of the irregular ectodermal mass with lens and a small vesicle attached, also showing double attachment to the roof of the pharynx. $\times 90$ diameters.

FIG. 61. Experiment DL₆₄. Optic vesicle transplanted anterior to the otic vesicle. Embryo killed 5 days after the operation. Section through irregular eye and lens. The lens is very irregular, a portion of it in the optic cup shows formation of lens fibers. The outer pole of the lens is very irregular and is connected with the ectodermal masses *a, a*. $\times 90$ diameters.

FIG. 62. Experiment DL₆₄. Section through lens mass 3 sections 30μ anterior to the above showing very irregular portion of lens connected with irregular ectodermal strands. The strand passing towards the ectoderm was probably at one time connected with the inner layer. $\times 180$ diameters.

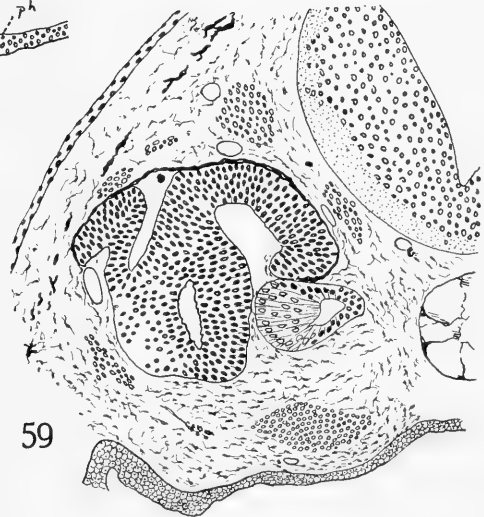
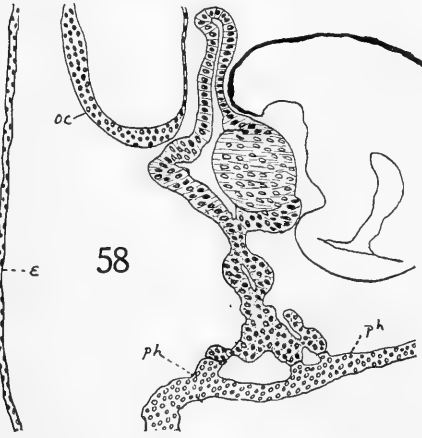
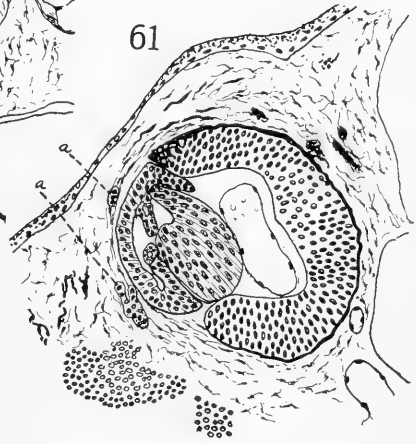
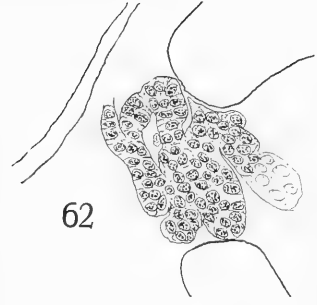
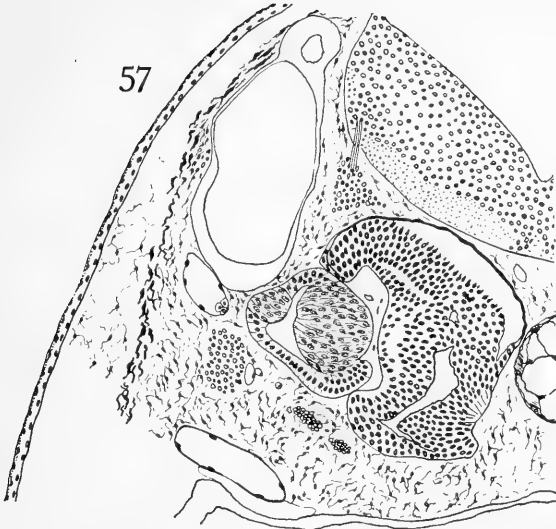


FIG. 63. Experiment DL₅₉. Optic vesicle transplanted anterior to otic vesicle. Embryo killed 4 days after the operation. Section through transplanted eye and lens. They are about as large as normal and the optic cup is as far differentiated. The lens is not so far differentiated as normal and is irregular, showing at its caudal end evidence of attachment to the ectoderm (see Fig. 64). $\times 90$ diameters.

FIG. 64. Experiment DL₅₇. Section through caudal end of lens, edge of optic cup, and ectoderm. The latter shows place where lens was attached. A few sections caudal to this one an ectodermal process continues off from the lens into the mesenchyme. $\times 90$ diameters.

FIG. 65. Experiment DL₅₇. Section through normal eye and lens.

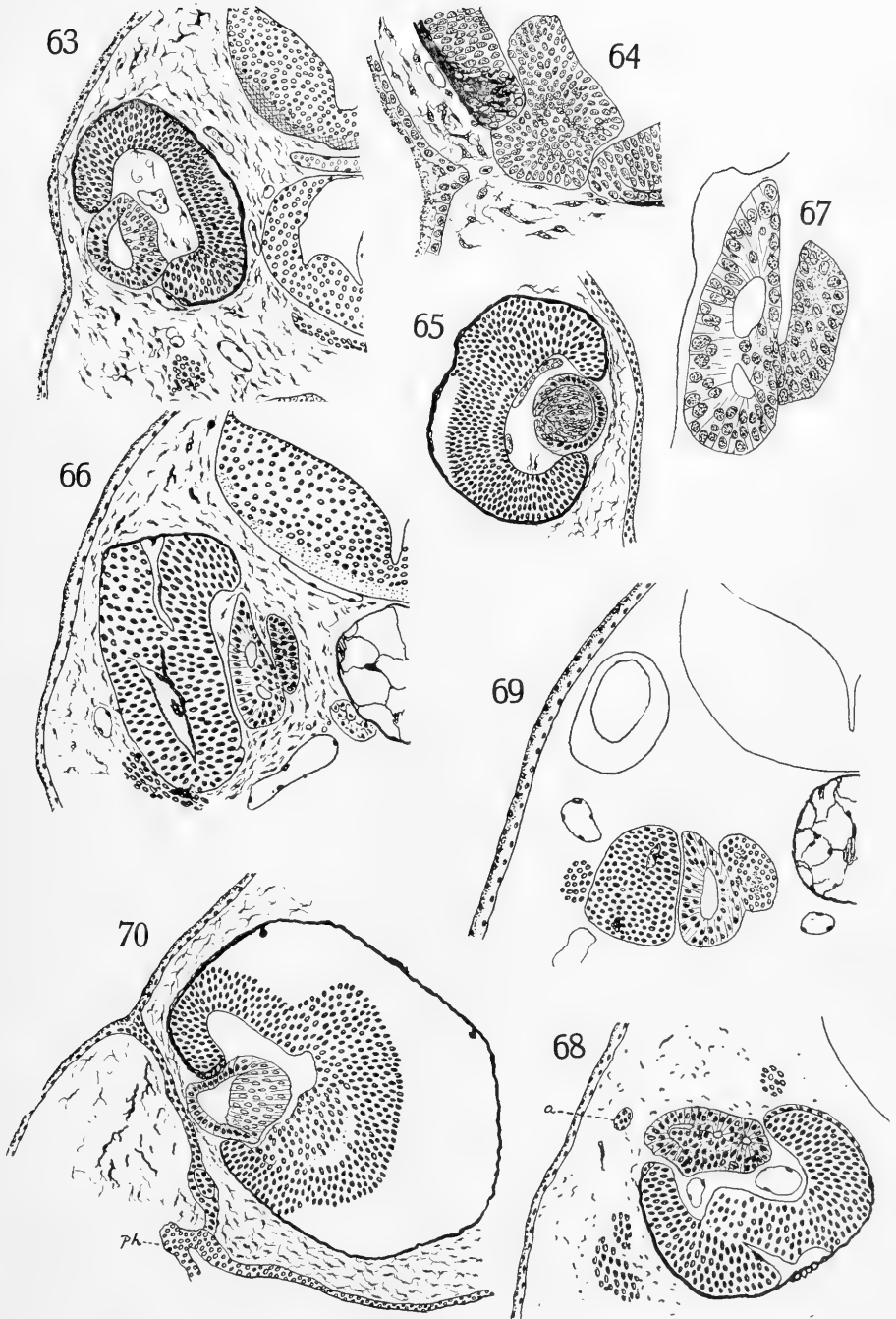
FIG. 66. Experiment DL₄₀. Embryo killed 4 days after the operation. Section caudal to the otic vesicle through transplanted eye, etc. On the median side of the irregular eye is an irregular mass of ectoderm with two cavities. $\times 100$ diameters.

FIG. 67. Experiment DL₄₀. Ectodermal mass from the above section showing beginning transformation into lens on the side towards the retinal portion of the eye. $\times 200$ diameters.

FIG. 68. Experiment DL₄₅. Embryo killed 4 days after the operation. Section through transplanted eye which lies caudo-ventral to the otic vesicle. In the cavity of the irregular eye is an irregular mass of ectoderm containing several cavities and small pigment masses. There are only slight signs of its differentiation into a lens. The ectoderm in this region was evidently injured, a loop of ectoderm cut in section at *a* crosses over the region of the eye. $\times 100$ diameters.

FIG. 69. Experiment DL₂. Embryo killed 3 days after the operation. Section through transplanted eye which lies ventral to the otic vesicle. On the median side of the transplanted eye is a large irregular mass of ectoderm and the part in contact with the eye seems to be undergoing transformation into lens tissue. $\times 100$ diameters.

FIG. 70. Experiment DL₆₅. Optic vesicle transplanted anterior in the otic vesicle. Embryo killed 4 days after the operation. Section through transplanted eye and lens. In this embryo an ectodermal strand extends from the ectoderm to the pharynx, only a small portion of it was in the section, the rest of it has been projected into the section from the neighboring sections. The lens has probably arisen from this strand and there is a slight indication on the process of the place where the lens was pinched off. *Ph*, pharynx wall. $\times 90$ diameters.



OBSERVATIONS ON THE ORIGIN OF THE PAIRED LIMBS OF VERTEBRATES.

BY

RAYMOND C. OSBURN.

From the Zoölogical Laboratory of Columbia University.

WITH 5 PLATES.

Perhaps in no field of Vertebrate zoölogy have two diametrically opposite views been more steadily and skillfully maintained than in that which forms the subject of the present paper. Disregarding the earlier speculations in regard to the origin of the paired limbs, we may date the real work in this field from the appearance of Gegenbaur's first paper on the subject in 1865. This prolific student adopted the earlier suggestion of Owen that the limb girdles correspond to misplaced gill-arches and proceeded to defend this view with such vigor and resource that it was for many years the accepted hypothesis. At the same time the unpaired fin skeleton was held to be a derivative of the axial skeleton. These views were steadily maintained by Gegenbaur through thirty years of investigation, indeed, up to the appearance of his last paper on the subject in 1895. His students have also contributed much to the support of the hypothesis.

Opposed to the gill-arch theory (Gegenbaur theory, archipterygium theory) is that known as the fin-fold, or lateral-fold theory, which postulates a similar origin for the paired and unpaired fins as local outgrowths from the body-wall, independent of gills, axial skeleton, or any other structures. This view was first developed in the researches of Thacher, 77, Balfour, 78, and Mivart, 79, all working independently, and has become pretty generally accepted by zoölogists, other than those of the Gegenbaur school. Very much has been added to the support of the theory since it was first propounded, but, in spite of the exploitation of anatomical, palæontological, and embryological fields there have always remained numerous ambiguous points, the interpretation of which depended on the attitude with which they were approached. That the problem was not fully settled by the older investigators witness at least

a dozen papers dealing more or less directly with its various phases appearing since 1900.

The work of the gill-arch theorists has been confined chiefly to drawing comparisons between the skeleton of the paired limbs and that of the gills, to studying the possibilities of migration in the paired fins, and to searching for connecting stages between the branchial and pectoral regions. But the study of the unpaired fins they have almost ignored. The studies of the fin-fold theorists, on the other hand, have been particularly directed toward the similarities between the median and paired fins and have aimed to prove the local and metameric development of the paired limbs. To the fin-fold theory the chief obstacle has been the fact that the paired limbs possess girdles while the median ones do not, a difficulty which the adherents of the gill-arch theory have considered insurmountable. For the latter theory the most important stumbling-blocks have been: the lack of any intermediate stages between gill and fin, the absence of proof of migration sufficient to account for the position of the pelvic fins, and the difference in position between the gill-arch in the gut wall and the fin girdles in the body-wall.

The various fields of anatomy, palæontology, and embryology have all been gleaned by various investigators for evidence to bear upon the problem and all have proved fruitful, though lack of more complete knowledge has at times caused apparent conflicts. The anatomical side of the question has been most thoroughly worked and our knowledge of the facts may be said to be fairly complete. Unfortunately, however, anatomical evidence is not the most conclusive in the study of phylogenetic questions, and in the structures of the fins the adherents of both theories find what they consider good evidence in favor of their views.

Palæontological data would obviously be of inestimable value in deciding doubtful points, if not the entire problem, but the evidence from fossils may always remain fragmentary and even conflicting. The Cladodont sharks are without doubt the oldest fossil forms which give us any light on the structure of the limbs, and they agree in possessing a fin-fold type of fin. Cladoseleche, from the Devonian, is particularly enlightening in this respect, but the gill-arch theorists have consigned Cladoseleche to the limbo of an aberrant group (Braus, 04). On the other hand, the Permian Pleuracanthus, while regarded by the gill-arch theorists as convincing evidence on their side of the question, is rejected by the adherents of the fin-fold theory. There seems no absolute anatomical reason why the pectoral fin of the modern shark might not have been derived (by different methods, to be sure) from either the biserial

type of *Pleuracanthus* or the fin-fold type of *Cladoselache*, but the question is, from which did it evolve? In taking up this question we have to consider not only the type of fin but also the possibilities of the animal as an ancestor of modern sharks. While *Cladoselache* itself may not have been the progenitor of modern sharks, the group to which it belongs is of such a generalized type that, as Dean points out (95), almost any later group of fishes might have arisen from it. In fact, the *Cladoselachidae* approach more nearly to the ancestral condition of true fishes than any other group of which we have sufficient knowledge to form a judgment. This simplicity is coupled with an antiquity much greater than that of the *Pleuracanthidae*. In regard to the latter family it may be suggested that the nuchal spine, the condition of the dermal skeleton, the character of the two anal fins, and the diphycceral caudal fin, remove it at once from our consideration as a possible ancestor of any known type of fish. Concerning the diphycceral caudal it is worthy of remark that all the oldest fishes known (*Pleuropterygidae*, *Acanthodidae*, and *Diplacanthidae* among the sharks, the most ancient *Dipnoi* and *Ganoidei*, as well as the fish-like *Heterostraci*, *Anaspida*, *Antiarcha*, and *Arthrodira*, all older than the *Pleuracanthidae*), had the heterocercal condition developed, so that, as far as we are able to judge from palæontology, heterocercy seems to be the primitive condition in the *gnathostomata*. The diphyccery of *Pleuracanthus* must then have been secondarily acquired, as it seems to have been in the *Dipnoi* (Dollo, 92, 93). Therefore, taking into consideration all the facts concerning these two ancient types of fishes, and bearing in mind that the *Acanthodidae*, *Diplacanthidae*, and *Pleuropterygidae* (all much older than the *Pleuracanthidae*), agree in having a fin-fold type of fin, we are led to believe that the evidence from palæontology is distinctly favorably to the fin-fold theory. It may be well to add here that even if the *Pleuracanthus* type of fin were proved to be primitive the branchial origin of the fins would still be unproved, for the fins of *Pleuracanthus* are no more gill-like than those of modern sharks. It would not at all preclude the possibility of the local origin of the fin structures, which is after all the most important contention of the fin-fold theorists.

In regard to the embryological evidence it may be remarked broadly that the recent investigations tend to show that in early development there is scarcely a point in which the paired and unpaired fins are not in perfect agreement. The only important exceptions, the girdles and the trapezius muscle, have, I believe, a satisfactory explanation as secondary rather than primary characters. It was the original intention of the

writer in taking up the present studies, to discuss thoroughly the embryological work on the origin of limbs, but the recent able papers of Professor Braus, though taking an opposite view of the question, have dealt so thoroughly with the literature as to make such a discussion no longer necessary. In May, 1906, the writer paralleled the arguments of the fin-fold and gill-arch theorists as a preliminary to the present paper. In the month following there appeared the admirable paper of Professor Goodrich, 06, in which he arrives at conclusions very similar to my own, based chiefly upon a study of *Scyllium*. The present writer has examined especially *Cestracion* and *Chlamydoselachus*. However, under the belief that embryological evidence from the last two forms mentioned should have special consideration on account of their primitive position among the genera of *Selachii*, and for the reason that certain heretofore unpublished facts have been brought to light during the pursuit of my studies, the publication of my results seems desirable.

I may state at once that as far as my own studies overlap those of Goodrich they are entirely corroborative of his work, and where they do not cover the same ground they are quite confirmatory of the fin-fold point of view. It should be said that our work was carried on in parallel lines and without any knowledge of the other's research. This, in itself, must add weight to our results. As will be seen, the evidence indicates strongly that the unpaired fins have originated as external structures entirely independent of the axial skeleton, and the remarkable similarity of the paired to the unpaired fins in mode of development can only be explained on the ground of similarity of origin. The failure of Braus, 04a, to find in *Spinax* any marked similarity between these fins is explicable only under the supposition that he did not make a careful comparison of the earlier stages in the development of the various component structures of these organs, or that he did not possess the proper material for such comparison. As a matter of fact, the unpaired fins of *Spinax* are very much concentrated (the anal is entirely wanting) and the early stages of development seem to be passed through much more rapidly than in *Cestracion*, though the dorsal fins of the latter species seem, in the adult, to show nearly as much concentration as those of *Spinax*.

Zoölogists generally agree in holding that the sharks approach more nearly than any other group the original condition of the gnathostome vertebrates, and it is to this group that we naturally look for embryological evidence bearing on the origin of the paired limbs. Naturally, also, we look for the best embryological evidence as to the origin of fins among those sharks which on anatomical and palæontological grounds

are to be regarded as most primitive. In this respect the writer has been most fortunate in having at his disposal a very complete series of embryos of the cestraciont shark *Heterodontus japonicus* Duméril,¹ and a number of embryos of the Notidanid shark *Chlamydoselachus anguineus* Garman, 85-86. Together with this material I have had for comparison many stages of Spinax, Mustelus, and Torpedo. Professor Bashford Dean has very generously placed this valuable material at my disposal, and I am indebted to him also for many kind suggestions and criticisms.

Certain observations have been made by various students of the fins and corroborated to such an extent that they may be considered as points definitely settled in selachian embryology, especially since they are not disputed by the followers of either the gill-arch or the fin-fold theory. Such are:

I. Each fin at its inception consists of a longitudinal fold of the epidermis. This has been observed by all students of shark embryology from Balfour, 78, down to the present. It may be added that the same observation holds almost universally among fishes, the few exceptions being among Teleosts whose limb structures we have reason to believe are highly specialized.

II. This fold of the epidermis is soon invaded by mesenchyme cells which establish a dense plate or mass of cells (mesenchyme plate or mesenchyme thickening) lying partly within the epidermal fold and partly within the body-wall. (Balfour, 78, 81; Dohrn, 84; P. Mayer, 86; Mollier, 93; Rabl, 97, 01; Braus, 04b, and others.) So much at least is beyond question. The exact origin and fate of the mesenchyme plate will be discussed later.

III. The fin-fold with its mesenchyme plate is next provided with segmentally arranged muscle-buds from the dorsal and ventral ends of the neighboring myotomes. This ingrowth in the paired fins is similar to that in the median fins. (Balfour, 78, 81; Dohrn, 84; P. Mayer, 86; Mollier, 93; Rabl, 97, 01; Braus, 98, 04a; Goodrich, 06, and others.)

IV. In the embryonic fin, whether paired or unpaired, there are abortive muscle-buds which degenerate without entering the fin. These are found in the posterior as well as in the anterior margin of the fin. In the paired fins these rudiments have been traced by Dohrn, 84; Mollier, 93; Braus, 98; Rabl, 01, and Goodrich, 06, and in the median fins by Dohrn, 84; Paul Mayer, 86, and Goodrich, 06.

V. The most anterior and most posterior muscle-buds which enter the

¹Throughout this paper *Heterodontus* is referred to as *Cestracion*, the older name by which the genus is usually known among morphologists.

fins, paired and unpaired alike, are compelled to reach out of a direct position (*i. e.*, out of the transverse plane of their myotomes) in order to get into the fin-fold. That is to say, the fin base is shorter at this period than the combined length of the myotomes concerned in supplying muscle-buds to the fins. This condition has been carefully discussed by Mollier, 93; Braus, 98, and Goodrich, 06, in the paired fins, and by Paul Mayer, 86, and Goodrich, 06, in the case of the unpaired fins. The writer has also observed it to be true for *Cestracion* and *Spinax* in both paired and median fins, 06b.

VI. The nerves which supply the paired and unpaired fins arise segmentally in the same manner as external rami of the dorsal and ventral branches of the spinal nerves. (Balfour, 78, 81; P. Mayer, 86; Mollier, 93; Braus, 98, 01; Osburn, 06b; Goodrich, 06.)

VII. The blood-vessels which supply the various fins all arise in a similar manner. They are all branches of body-wall blood-vessels which take their origin on the dorsal side of the dorsal aorta.

VIII. The procartilaginous anlage of the fin skeleton arises in the same way in all fins, differentiating out of the mesenchyme plate; and, when basals are present, as in the paired fins of all sharks and the dorsal and anal fins of many, the rays and basals (and the girdle in the paired fins) form one continuous structure. (Balfour, 78; Mollier, 93; Ruge, 02; Braus, 04a; Osburn, 06b; Goodrich, 06.)

IX. As the continuous procartilaginous anlage of the fin skeleton becomes differentiated into cartilage, the formation of joints takes place (Ruge, 02), with the result that the elements of the adult fin are defined. The process is identical in all the fins, but the amount of separation varies greatly according to the species, depending probably upon adaptation, *i. e.*, as to the special way in which the fin comes to be used.

X. The sequence of development of the various structures entering into the formation of the fin is the same in all cases, *viz.*:

(a) The epidermal fold is raised.

(b) The mesenchyme pushes its way into the fold and forms the mesenchyme thickening.

(c) The muscle-buds migrate into the fins accompanied by their respective branches of the spinal nerves.

(d) The continuous procartilaginous anlage of the fin skeleton becomes differentiated *in situ* out of the thickened mesenchyme (this includes also the girdles of the paired fins).

(e) The chondrification of the skeleton takes place, with the formation of joints and the consequent separation into skeletal elements.

XI. Collector nerves and plexuses are known to occur commonly in both paired and unpaired fins, and, though usually more marked in the anterior margin of the fin, they are of frequent occurrence in the posterior as well. (For the paired fins, Davidoff, **79**; Mollier, **93**; Punnett, **00**, **01**; Braus, **98**, **04a**; for the unpaired fins, P. Mayer, **86**; Goodrich, **06**.)

XII. The bases of the paired fins and of the dorsal and anal fins become concentrated during development. (Mayer, **86**; Mollier, **93**; Goodrich, **06**.) This is least marked in the case of the caudal fin and most marked in the pectoral fins, and between these extremes there exist all intergradations in anal, dorsals, and pelvics. Measurements show that the base of the pelvic fin of *Chlamydoselachus* is comparatively longer than that of the dorsal fins of *Cestracion* or *Spinax*.

XIII. All types of fins may migrate or be displaced during development. Such migration is always of slight degree, though often measurable, and may be in either direction (Dean, **02**; Punnett, **04**).

XIV. Ceratotrichia, or horny rays of the fins, develop in both paired and unpaired limbs and occur in no other part of the body. They are equally ancient in either type of fin (Goodrich, **03**; Osburn, **06b**), and appear at about the same time in ontogeny (Goodrich, **03**).

The foregoing points may be considered as already decided. Upon the points which are still under consideration, the writer desires to present the following data and discussion:

FUSION OF MUSCLE-BUDS.

Muscle-buds may fuse during their early development in the unpaired as well as in the paired fins. Such fusion or blending was first described and figured in the pectoral of *Torpedo* by Mollier, **93**. Later, Fürbringer, **02**, and Braus, **04a**, used this fact, in opposition to the fin-fold theory, as evidence against the metamerism of the adult muscles of the paired fins, and so, also, of the corresponding skeletal rays. My own work on *Cestracion*, however, shows that fusion of muscle-buds is not confined to the paired fins, but, on the contrary, may occur in any of the unpaired fins. Hence, as I have already indicated, **06b**, any argument against the fin-fold theory based on such ground is altogether futile. In *Cestracion* fusions were found in all of the unpaired fins,—even in the caudal, though more sparingly here,—and the nature of the blending is exactly similar to that in the paired fins. Fig. 17 shows such a fusion in the anal fin of a 35 mm. *Cestracion* embryo. A comparison with Mollier's figure (**93**, Taf. III, Fig. 15) of this condition in

the pectoral of *Torpedo* will indicate the similar nature of the fusions even in such distantly related forms.

As to the later fate of these blended muscle-buds there remains yet some uncertainty. Fürbringer, **o2**, and Braus, **o4a**, have maintained that from the time of fusion of the buds the muscles are dysmetameric and their innervation polyneurous. This supposition as to the secondary polyneury of the paired fins is by no means borne out by the recent experiments of Goodrich, **o6**. The latter investigator has tested physiologically the exact distribution of the branches of the spinal nerves which enter the fins, and he finds that in *Raja* each motor nerve is distributed to one and only one radial muscle, and that under stimulation there is no spreading of the impulse such as would be the case if polyneury existed. The confused plexus at the base of the fin must then, as Goodrich observes, embrace only the sensory nerves, while the motor nerves pass through the plexus without losing their individuality. The muscles after fusion must, therefore, again separate without losing their haploneurous condition, at least as far as the motor nerves are concerned.

Goodrich has even ventured the suggestion (**o6**) that this fusion is not a fusion of muscle-buds involving embryonic muscle-tissue at all, but that what has been supposed to be such a blending is merely the anlage of the sensory nervous system which, arising in this region, gives the appearance of the fusion of muscle-buds. We cannot subscribe to this opinion, however, for, by actual observation, the muscle-buds at an early stage may be seen to be in contact and that with such clearness as to leave no doubt as to the interpretation. Such fusions are of frequent, but very irregular, occurrence in *Cestracion*, and there is no apparent order in their arrangement. They are most abundant in the anal fin in which the adult muscles are blended into a confused mass, and rarest in the caudal where the muscles of the adult are very regularly arranged. Moreover, blended muscles certainly do occur in the older embryo (Fig. 18, of the condition in the second dorsal fin of a 58 mm. embryo of *Cestracion*), and remain blended in the adult. In this case the bases of the muscle-bands no doubt remain fused because of the great amount of concentration at the base of the fin. The condition in the anal is similar, only carried much farther in concentration.

How are these fusions to be explained? Not, as Braus, **o4a**, concludes, for the purpose of accomplishing the polyneury of the fin muscles, since Goodrich's experiments show that the muscles are not so innervated; and not, as Goodrich, **o6**, suggests, that the apparent fusions are rudiments of the developing sensory nerve plexus, for blending actually

occurs and remains in the adult. Rather they seem to the present writer to represent accidental points of contact of neighboring muscle-buds which in their embryonic condition fuse merely because of the juxtaposition of similar cells, and which may later become separated by the growth of intervening connective tissues forcing the muscles farther apart, and by the development of the embryonic muscle-cells into fibers.² As we have seen, they sometimes remain in contact. That these fusions are merely accidents of development is indicated by the fact of their sporadic and variable occurrence. They do not occur between the distal ends of the muscle-bands where they are farther separated, they are most abundant where muscle-buds are crowded together (*e. g.*, anal of *Cestracion*), and least abundant where there is least crowding (*e. g.*, inferior caudal of *Cestracion*). In the paired fins they are most abundant near the ends of the series of muscles and they are sometimes entirely wanting between the buds at the middle of the fins. Moreover, there is no uniformity in the extent of the fused portions.

DISCREPANCIES BETWEEN MUSCLES AND CARTILAGINOUS FIN RAYS.

Discrepancies between the muscles and cartilaginous fin-rays with regard to arrangement may be present in unpaired as well as paired fins. Braus, 04a, first carefully worked out the discrepancy as it exists in the pelvic fin of *Spinax*, and he at once interpreted it as an argument against the origin of the paired fins as metameric structures. That such discrepancy may exist in the paired fins is probably beyond question. This condition, however, it now appears, is not confined to the paired fins and therefore the fact does not bear against the fin-fold theory, since the median fins, which show an equal amount of discrepancy, are by the gill-arch theory assumed to be strictly metameric in origin. Fig. 15 shows this discrepancy in the second dorsal fin of a 53 mm. embryo of *Cestracion*. It is in all respects similar to that which Braus figures in the pelvic of *Spinax* (04a, Taf. XIV), and is of even greater extent.

If we inquire into the cause of such discrepancy as above indicated I am convinced that sufficient reason can be found in the adaptation of the fin to meet the greatest mechanical needs. This explanation is given weight by the variation shown by different fins in the degree of concordance they exhibit. In some cases which I have examined,—*e. g.*, the pectoral of *Raja* and *Cestracion*, the anal and pelvic of *Chlamydoselachus*,

² A somewhat analogous process is found in the reopening of a portion of the Amphibian blastopore in the formation of the anus.

and the inferior caudal of most sharks,—there is a very close correspondence or concordance throughout the fin. In certain cases,—*e. g.*, the pelvic of *Spinax* (Braus) and the dorsals of *Cestracion*,—there is a concordance over a large portion of the fin and discrepancy over the remainder, while in the anal fin of *Cestracion* it would be a difficult matter to trace any exact concordance between the cartilage plates of the fin skeleton and the broad sheet of muscle derived from the intimate fusion of the muscle-buds. Thus, instead of weighing against the fin-fold theory, the facts of discrepancy only point more certainly to the close relationship between the paired and median fins.

MODE OF ORIGIN OF THE FIN SKELETON.

The origin of the mesenchyme cells which first occupy regions of the body in which the fins are later to be developed is already well known, thanks to the researches of Balfour, 78, 81; Boyer, 92; Rabl, 91, 97, and Mollier, 93. The concentration or thickening of this mesenchyme which gives rise to the fin skeleton begins in all fins just beneath the ectoderm at about the same time as the uplifting of the ectoderm to form the fin-fold. Figs. 1, 3, 4, and 5, show it in various fins. From this position the concentration extends outward with the growing fin-fold and inward until it covers all the region which the fin skeleton later occupies. The skeleton is derived from this concentrated mesenchyme,—not only the more externally situated fin-rays but also the basalia of the median and paired fins and the girdles of the latter as well. The change takes place by the direct differentiation of the mesenchyme tissue into the continuous procartilaginous anlage of the skeleton, and this later, by chondrification, becomes the definitive skeleton. In this process there are no topographic changes, the formation of joints taking place *in situ*. The concentration of the mesenchyme is from the first easily distinguishable from that which develops into the ordinary connective tissue. Figs. 1 to 6, inclusive, illustrate the difference in the appearance of the two kinds of cells. There can be no doubt that, ontogenetically, the earliest support of the fins in all cases is this concentrated mesenchyme which makes its appearance in and beneath the fin-fold long before the muscle-buds and nerves enter, and which becomes directly differentiated into the cartilaginous skeleton of the adult fin. There seems, therefore, no good reason for thinking that the evolution of the fin has followed any other course than this during its phylogeny.

THE INFERIOR CAUDAL FIN.

It is important to observe that the inferior caudal fin of *Cestracion* in its earlier stages agrees exactly with the other unpaired fins. This particular fin has always been a point of contention among students of the limb problem, since in the development of the definitive skeleton the rays in most cases arise in direct connection with the hamal spines of the axial skeleton, while in no other fin of any fish are the rays known to arise in this connection (with the one exception of the superior or epichordal portion of the diphyccercal caudal fin of *Lepidosiren* (Braus, **04a**, **04b**), the most highly specialized of the Dipnoi). There can be but three views in regard to this difference: 1st, The inferior caudal may be considered to have had an entirely different mode of origin from the other fins; 2d, it may be looked upon as the type of all the unpaired fins under the supposition that the others have lost their connection with the axial skeleton; or, 3d, that the connection of the rays of this fin with the axial skeleton has come about secondarily. Balfour and Parker, pioneers in vertebrate embryology adopted the first view, but no one since their time has suggested it. The second view has been adopted by the adherents of the gill-arch theory, following Gegenbaur's assertion that the cartilages of the dorsals are only modified spinous processes, "losgelöste und selbständiger gewordene Dornfortsätze." Professor Braus attempts (**04a**) to support this hypothesis by the observation on the superior caudal fin of *Lepidosiren* already alluded to, making this dorsal connection an intermediate step between the inferior caudal and the dorsals. This sort of argument seems to us hardly warranted when the high specialization of *Lepidosiren* and the wide phylogenetic gap separating the modern Dipnoi from the Selachii are considered. The third view, viz., that the unpaired fins have all had a similar, external origin, and that the inferior caudal has become secondarily attached to the axial skeleton for mechanical reasons, is the one adopted by the fin-fold theorists. This view was advanced by Dohrn, **84**, and strongly supported by Paul Mayer, **86**, who found in the anterior part of the inferior caudal fin the evidence of a separate origin of the rays of this fin,—“ferner wäre vom Knorpel noch zu bemerken, dass er wie bei allen anderen Flossen erst secundär mit der Wirbelsäule in Verbindung tritt.” Now, *Cestracion*, at an early stage in the development of the inferior caudal, offers a very striking confirmation of the view that this fin has arisen in an external manner similar to the other unpaired fins. Fig. 3 shows a section through the fin at a time when the mesenchyme plate from which the fin rays develop is entirely separated

from the region of the axial skeleton. The latter, moreover, has not at this stage become noticeable in the region of the hæmal spines. The mesenchyme of the fin continues to develop toward the axial region while that of the latter becomes more concentrated and at last the two regions are united. When the rays become evident by the differentiation of the mesenchyme they arise *in situ* and do not grow out³ of the hæmal spines. The same statement holds good for the development of the rays and basals of all fins, paired and unpaired,—all arise by differentiation in their proper position from the mesenchyme plate.

The other unpaired fins not only resemble the inferior caudal in having an external origin for the mesenchyme plate, but they never originate in contact, much less in continuity, with the axial skeleton. In case spines are present, as in Spinax, Cestracion, etc., the spine secondarily comes into contact with the vertebral column for support. That this attachment is secondary is admitted even by the gill-arch theorists. Species, among the lower sharks, which do not possess fin spines usually have the fin skeleton widely separated from the axial skeleton (Notidanus, Chlamydoselachus, Mustelus, Scyllium, Lamna, etc.). Figs. 19 and 16 are camera drawings from Van Wijhe preparations of the dorsal and anal fins of Chlamydoselachus showing the relation to the vertebral column in an embryo of 225 mm. In Cestracion the large basale of the anal fin comes nearly or quite into contact with the ends of the hæmal spines. A study of the development of this form, however, shows plainly that the basale in question arises earlier than, and entirely independent of, the hæmal spines. Figs. 12 and 13 are camera drawings of vertical sections through the anal fin of a 40 mm. embryo, illustrating the independent origin of the fin skeleton.

PAIRED FINS CONTRASTED WITH GILLS.

The followers of Gegenbaur have always asserted the similarity of the paired fins to the gills, and recently K. Fürbringer, 03, and Braus, 04a, have renewed the attempt to compare these structures, the former ana-

³ As I have elsewhere (06b) pointed out, the terms "grow out," "auswachsen," etc., are entirely misleading when applied to the development of the fin skeleton. The muscle-buds may be rightly said to grow out into the fin, since by internal development the tip of the bud is moved forward. Its progress is marked by the disarrangement of the connective tissue and cells of the mesenchyme plate as the bud forces its way by such growth into its position in the fin. Figs. 5 and 6 illustrate this disarrangement and show how the cells are pushed out of the way. There is nothing comparable to this in the development of the fin skeleton since the rays elongate by the continued differentiation of mesenchyme cells at their ends.

tomically, the latter embryologically. The latter author, in particular, insists that as good a comparison can be drawn with the gills as with the unpaired fins and in the same points, viz.:

- “ 1. Isolierte Radien.
- “ 2. Basalia als ontogenetisch einheitliche Anlagen.
- “ 3. Ausbildung uniserial angeordneter Radien: Monostichopterygium.
- “ 4. Ausbildung biserial angeordneter Radien: Distichopterygium.”

It may be true that such arrangements of skeletal parts are to be found in the gills as well as in the fins, but even in the skeleton the weight of evidence is against such comparison and when we attempt to carry it to the other structures all similarity ceases. The following points will serve to indicate on what a slender basis rests the comparison of gill with fin:

1. The concentration of mesenchyme cells which gives rise to the gill skeleton originates internally in contact with the pharyngeal endoderm and spreads outward, while that from which the fin skeleton arises originates externally in contact with the ectoderm and develops inward. Figs. 1 and 2 show this for the fins.

2. The paired and unpaired fins arise external to the blood system, *i. e.*, to the main blood-vessels, while the gill-arches lie internal to these (Fig. 7).

3. The paired and unpaired fins are external to the coelom, the gill-arches internal.

4. It is worthy of note that the structures in the branchial series which resemble the fin (the arch excepted) are confined to the hyoid whose function as a gill is somewhat degenerated, and the structures are unquestionably the result of concentration and reduction. But the hyoid arch with its appended structures is most certainly not becoming a fin, so of what avail is the comparison?

5. As shown elsewhere, the pectoral girdle, though somewhat similar in shape and position to the gill-arches, is not in series with them.

6. The radial structures of the gill are always arranged in a vertical series (this is equally true of those of the hyoid arch above mentioned), while those of both paired and median fins are always in longitudinal series.

7. The fin-fold, which is the earliest indication of the fin, in all cases extends longitudinally, *i. e.*, parallel with the axis of the body. The gill membranes arise in a vertical position, at right angles or nearly so to the body axis.

8. The muscles of the median and paired fins are in all respects similar in origin while those of the gills are entirely different.

9. The nerves which supply the paired and unpaired fins are of the same sort, all branches of the spinal nerves (that of the trapezius muscle excepted) while the gills are innervated entirely by visceral nerves.

10. The blood-supply of all the fins is the same in character, *i. e.*, all are supplied by those vessels which go to the body-wall of that particular region. In the pectoral fin at the earliest stage there is not the slightest indication of any other blood connection than that of the brachial artery. Fig. 14 shows the origin of this vessel in the manner characteristic of all arteries supplying the outer wall of the trunk region, in a young embryo of *Cestracion*, in contrast with the termination of the last efferent branchial artery.

11. Ceratotrichia, or horny fin-rays, are always present in both paired and unpaired fins of all sharks, even the most ancient (Dean, 94; Goodrich, 03; Osburn, 06b), and are unknown in gills.

FIN GIRDLES AND GILL-ARCHES.

The gill-arch theorists still maintain (Fürbringer, 02; Braus, 04a, 04b), as a necessary foundation stone of their theory, that the limb girdles are serially homologous with, and in every respect the counterpart of, the gill-arches. Let us examine this so-called serial homology. It is true that the pectoral girdle does originate close behind the gill region, and that in form it is similar, as any structure situated in the body-wall as the pectoral girdle is, must of necessity be arciform. But here the evidence in favor of the serial homology ceases. Examining the evidence on the negative side of the question, we find in the first place that the pectoral girdle makes its appearance quite ventral to the gill region. Braus's own figures of *Spinax* show that the anlage of the girdle is situated almost its whole length below the gill region (Braus, 04a, Fig. 1, Taf. XIII), and the accuracy of this illustration I can attest from my own studies on a 20 mm. embryo of *Spinax*, as well as from my *Cestracion* studies. Fig. 7 shows a camera drawing to the same scale, of a section through the fourth gill-arch and one through the pectoral girdle at an early stage of the latter before the dorsal portion makes its appearance. The dotted lines connect similar points. The contrast is obvious.

In the second place the more external position of the pectoral girdle prevents any homology with the gills. Fig. 7 illustrates this contrast. The gill-arch is in contact with the pharyngeal endoderm and is internal to the blood system while the pectoral arch comes into contact with the

ectoderm and lies not only external to the body cavity but also to the larger blood-vessels. This condition is continued into the adult where a portion of the last gill-arch is overlaid by the pectoral girdle.

MIGRATION OF THE PECTORAL ARCH.

The question of the migration of the paired limbs is a vital one for the gill-arch theory since the origin of the limb girdles from gill-arches involves their translation from the branchial region. That a certain amount of shifting of position may occur during the development of the paired fins no one will deny, but that the same sort of shifting may also occur in the growth of the unpaired fins is equally true. Such migration is always comparatively slight, and it may take place either forward or backward. Moreover, the shifting of the paired fins is correlated with that of the unpaired fins (Dean, 02; Punnett, 04) and with that of the center of gravity of the body of the developing embryo (Dean, 02). With regard to the supposed early backward migration of the pelvic described by Braus, 98, the writer has already (06b) suggested a different interpretation, and Goodrich, 06, has stated, as a result of careful observations on Scyllium, that the facts of development will not bear the interpretation given by Professor Braus. The abortive muscle-buds anterior to the pelvic fin cannot, then, represent the path over which the pelvic fin migrated to its present position, but, like those in front of and behind the unpaired fins and even behind the pelvic itself, they represent muscles which once functioned before the base of the fin became as constricted as in recent adult sharks. The attempt to explain the abortive muscle-buds behind the pelvic fin as due to a secondary forward migration of that member (Punnett, 00) is a *reductio ad absurdum* of the migration hypothesis. In their earlier appearance the anterior abortive muscle-buds merely follow the law of all such serial structures of the body, in that the most anterior are the first to arise.

The presence of a collector nerve in the anterior part of the pelvic fin cannot be considered as evidence of migration of the fin since such structures are now known to occur in the posterior part of the same fin and in the unpaired fins, both posteriorly and anteriorly. (Cf. Mayer, 86; Punnett, 00; Braus, 04; Osburn, 06b; Goodrich, 06.)

Forward migration of the pectoral fin.—By the biometric study of a very complete series of embryos of Cestracion, Dean in 02 showed that the pectoral fin as a whole shifts forward during development, instead of backward as the gill-arch theory would require. The present writer has investigated the internal structures of the pectoral fin in this form and

finds the shifting concerns not only the distal portion but that even so deep-seated a structure as the pectoral girdle is involved in the forward migration. Fig. 8 shows the relation of the pectoral girdle to the last two gill-arches at the time of the first differentiation of the dorsal part of the girdle from the mesenchyme into procartilage. Figs. 9 and 10 show later conditions in embryos of 35 mm. and 53 mm., respectively. A comparison of these figures shows that at its earliest appearance the girdle is removed from the gill region by a space much greater than that which separates consecutive gill-arches, and that as development proceeds the space is gradually lessened. In the adult *Cestracion* the girdle and last gill-arch have shifted past each other to the extent that a portion of the gill-arch lies internal to the girdle. Could any further evidence be desired to disprove at once the hypotheses of the serial homology of these structures and of the origin of the paired fins by migration? One more point, however, demands our attention.

The trapezius muscle.—This muscle, which is derived from the visceral musculature and is supplied by visceromotor nerves, is attached to the anterior surface of the scapular portion of the pectoral arch. With this sole exception, all the muscles of the fins, paired and unpaired, are derived from myotomes and innervated by spinal nerves. This one exception, however, has been the occasion of much controversy, for it is considered by the adherents of the gill-arch theory to be a relic (“Die alten Relikte des einstmaligen Kopfmuskelsversorgung,” Fürbringer, 02) of the former visceral muscle supply of the pectoral arch when it was a gill-arch and before it was abstracted from the branchial region (according to the hypothesis) by the action of the spinal muscles (Fürbringer, 02; Braus, 04a). From the contrast which we have already drawn between the gills and fins it is evident that such an explanation of the trapezial connection is not the correct one. But, aside from such inference, I think we can prove the case on its own merits. The studies of E. Ruge, 02, and Braus, 04a, showed that in *Spinax* the first anlage of the pectoral girdle consists of the ventral portion only, from the region of the fin downward, and that the dorsal or scapular portion appears later. This I have been able to confirm in *Spinax* and to determine also for *Cestracion* (Fig. 7). Now, it is important to note that the trapezius muscle is attached to the dorsal or scapular part of the girdle while the ventral portion lies entirely within the region of somatic muscles. Furthermore, at the time when the scapular part of the girdle appears the trapezius has not yet grown backward from the branchial region to its point of attachment with the girdle, but a little later grows down and

comes into contact with it. In short, the first anlage of the pectoral girdle is wholly situated within the domain of somatic muscles and the trapezial connection is accomplished later by the dorsal growth of the girdle and the postero-ventral growth of the muscle. If the time element is at all trustworthy here (and we must believe so until it is proved otherwise) the above facts can only be interpreted to mean that the ventral portion of the girdle is more primitive than the dorsal, and, if this is true as the development indicates, then at one time the pectoral girdle consisted only of a ventral portion and was in somewhat the same condition as the pelvic girdle in most fishes to-day.

The discovery of this early condition of the pectoral girdle, I conclude, shows its complete distinctness from a gill-arch and places it in the same category with the pelvic fin. The gill-arch theorists have generally considered the pelvic fin as simplified from a condition still retained in the pectoral, or, in other words, as the more modified of the two,—a deduction necessarily following their assumption as to its mode of origin. But the facts indicate clearly that the pelvic fin represents the more primitive type, and that the pectoral fin passes through a similar stage of development and then progresses beyond this to the condition seen in all recent sharks.

The connecting links between the pelvic and unpaired fin skeletons have been so clearly presented (Thacher, 77, 78; Mivart, 79; Wiedersheim, 92; Regan, 04) as to need no particular comment here. I wish to bring up the case of *Chlamydoselachus* in this connection, however, because it has also a bearing on another point. The adherents of the Gegenbaur theory have tried to find some dorsal projection of the pelvic girdle to homologize with the scapular portion of the pectoral girdle, but the "*pars iliaca*" of Davidoff, 79, 80, cannot be considered, and the "*processus iliacus*" of Braus, 04a, must also be thrown out of comparison since it does not bear the same relation to the nerve foramen as the scapula does in the shoulder girdle. In *Chlamydoselachus* the pelvic girdle is a broad flat plate (see Fig. 20 from a camera drawing of a Van Wijhe preparation of a 225 mm. embryo) which serves also as a basale for about half of the rays of the fin. It is pierced by eight spinal nerves instead of a single collector. It has not the slightest indication, even in a 120 mm. stage, of any dorsal prominence whatever. Such a condition is impossible of explanation under the gill-arch theory, for the pelvic of *Chlamydoselachus* cannot by the greatest stretch of the imagination be made to homologize with a gill. In fact it resembles nothing so much as it does the flat, plate-like basalia of certain unpaired fins.

THE ORIGIN OF THE GIRDLES.

While upon this point it must be admitted that the evidence is not as complete as we would desire it to be, and while in a few respects it is even conflicting, yet, reviewing the whole matter carefully, the origin of the girdle may, I believe, be traced to the supporting elements of the fin.

The *raison d'être* of the girdle is, naturally, the necessity for a solid base in the soft body-wall, against which the remainder of the fin skeleton may brace itself. The rays of the unpaired fins may, when necessary, find support against the axial skeleton, but as the paired fins are situated support is impossible unless developed for that special purpose. Is such a development impossible of conception or unparalleled in morphology? For answer we will cite the extradigital cartilages which support the swimming membranes of the seal, the extension of the cartilage area, and the development of accessory digits in Cetacea and Ichthyosauria for the same purpose (Kükenthal, 90; Osburn, 06a) the calcar of the bat's wing, auricular and nasal cartilages, etc., etc., not to mention the visceral skeleton which must at some time have arisen *in situ* for the support of the gills.

Is there anything similar to this process in the unpaired fins? Undoubtedly, for in those forms in which, in the adult condition, the fin skeleton rests against the axial skeleton for support (Spinax, Cestracion, Acanthias, etc.), the embryonic fin skeleton is at first developed separately from the spinal column and by later growth comes into contact with it. In fact, in the unpaired fins of nearly all sharks there is more or less development of the proximal portion of the radials into basalia. The pelvic plate or girdle of Chlamydoselachus is certainly most similar to such basalia of the median fins. It serves as a direct support for half of the rays of the fin (without the intervention of any other cartilages), and the two girdles meet at the mid-ventral line for mutual support (Fig. 20), just as the basalia of certain median fins grow into contact with the axial skeleton for the same reason. The pelvic basalia or girdles are not in contact when they first appear but meet as development proceeds. The pelvic skeleton is not very unlike that of the anal if we suppose a similar amount of "fusion" to take place in the latter.

Is it a mere matter of coincidence that this simple condition of the pelvic fin should appear in a species which gives so many other evidences of great antiquity? We think not, especially when we take into consideration the evidence from Cladoselache (the oldest fossil shark in which the pelvic structures are known (see Dean, 94, Pl. VII, Fig. 2,

for figure of this fin), in which the pelvic fin skeleton is in a condition similar to the anal of *Chlamydoselachus* at present). Regan's important observations (04) on *Psephurus* are corroborative, as are also the earlier researches of Thacher, 77, 78; Mivart, 79, and Wiedersheim, 92.

An intermediate condition between the pelvis of *Chlamydoselachus* and that of ordinary sharks (such as *Scyllium*, *Spinax*, *Mustelus*, etc.) is seen in the other *Notidani* (*Hexanchus*, *Heptanchus*).

The pectoral girdle is of one type with very little variation through the whole group of sharks. In *Spinax* and *Cestracion*, and probably in all sharks, it consists, at the time when it first becomes evident in the mesenchyme, of a short bar lying next to the ectoderm. Its upper end is continuous with the primary basal of the fin and there is at this time no dorsal (scapular) portion. In this condition the pectoral girdle corresponds very closely to the pelvic girdle at the same stage of development. With later growth the dorsal part appears and the ventral end extends downward toward the mid-ventral line. The similarity of the two girdles is thus lost by the pectoral passing into a more specialized condition while the pelvic remains in a more primitive state.

Professor Braus has recently (04a) tried to show that the pelvic girdle is a degenerate structure in the adult, through the loss of a dorsal process which he homologizes with the scapular portion of the pectoral girdle, and therefore, of course, with the gill-arch. While we cannot doubt the accuracy of Professor Braus's observation as to the occurrence of such a dorsal process in *Spinax*, we cannot agree to the interpretation he places upon it. The writer has elsewhere, (06b) stated his objections to this view, which are, in brief: That the prominence in question is posterior to the nerve foramen, while the "scapula" is anterior in all sharks; that the lowest recent sharks known, the *Notidanidæ*, have no indication of such a prominence either in the adult or the young embryo; that the most ancient fossil shark of which the pelvic fin skeleton is known (*Cladoselache*) gives no indication of such a dorsal prominence. Whatever the aforementioned process in *Spinax* may represent, it certainly cannot be the vestigial homolog of the "scapula" unless the nerve foramen has shifted from the posterior to the anterior side of the process, and even then we should have the paradox of the most degenerate condition known in the shark limb occurring at once in the oldest fossil, and in the most primitive recent, sharks.

Concerning the discovery by Ruge, 02, and Braus, 04a,—and the writer has verified this discovery,—that in *Spinax* the first part of the procartilagenous anlage of the pectoral fin skeleton to appear is the girdle, it

must be urged that at a still earlier stage the mesenchyme thickening from which the fin skeleton arises has its origin next to the ectoderm within the developing fin-fold. In *Cestraçion*, according to the writer's observations, the first part to appear as procartilage is distal to the girdle and includes a portion of the primary basal and the proximal ends of the fin-rays in the forward half of the fin. From this point the process of differentiation spreads in all directions and soon the ventral half of the girdle makes its appearance. This is after the fashion which has been described by the older embryologists. Fürbringer, 02, and Braus, 04a, have used Molliers' figures (1893, Pl. III, Fig. 13, and Pl. IV, Fig. 16) to prove that in *Torpedo* a portion of the girdle is included in the first part of the skeleton which becomes evident. In this they are probably correct, but had they observed a little more closely they would have noticed that this portion of the anlage of the skeleton is not at all girdle-like in form but is merely an inward extension of the primary basal, while the real girdle or arch appears later growing out ventrally and dorsally from this region.

The apparent contradiction in the development of the pectoral fin girdle earlier or later than the more distal part of the fin skeleton is entirely cleared up when we go back to the previous stage in the formation of the skeleton. Here, as we have already seen, the concentration of skeletogenous mesenchyme progresses uniformly, as in all fins, from the ectoderm inward and forms the most primitive support of the fin-fold. Finally, after the muscles and nerves are all in position (the trapezius excepted), the fin skeleton appears in procartilage by differentiation of the mesenchyme in the position which it holds in the adult. The whole development of the pectoral and pelvic fins is just what we should expect for structures arising as local organs of the body-wall, and, except for the trapezial connection already explained, involve no other structures but the muscles, nerves, blood-vessels, and skeletogenous tissues of the immediate region in which they arise.

CONCLUSION.

It will be seen, in conclusion, that all of the important objections of the gill-arch theorists have now been met and answered. Nearly every condition observed in the paired fins has been shown to exist also in the median fins. The facts used by recent writers in defense of the gill-arch theory, viz., abortive muscle-buds, fusion of muscle-buds, migration of fins during development, collector nerves, origin of the skeleton as a continuous procartilaginous anlage, discrepancy of rays and muscles, and post-axial rays, are thus shown to have absolutely no weight in such

argument but merely indicate still more clearly the exceedingly close relationship which exists between the paired and median fins. The serial homology of limb girdles with gill-arches is shown to be impossible, and the trapezial connection can only be secondary. The branching cartilages observed in the hyoid region are to be regarded only as parallelisms,—produced probably by similar causes to those which have been operative in producing the fusion of rays to form basalia in the fins. Concentration or abbreviation of the region at base of the radii would seem sufficient to cause this in either fin or gill.

On the other hand, the manner of development of the pectoral girdle proves it to have been derived through a type similar to the pelvic, showing the latter to be the more primitive in form, while proof is not wanting to show the connection between basalia (girdles included) of paired and unpaired fins. The study of the earliest conditions in the formation of the inferior caudal places this fin on the same basis as the others and indicates the similar external origin of all. When we consider, in addition to the foregoing points, the exactly similar origin and development of the muscles, nerves, blood-vessels, and ceratotrichia in both kinds of fins, the case becomes so strong as to appear a certainty. I think we may safely conclude that the external or fin-fold origin of the paired limbs is clearly demonstrated, at least as fully as is possible until palæontology shall reveal the whole story by supplying the absolute serial stages in the evolution of these organs.

COLUMBIA UNIVERSITY,

March 9, 1907.

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DESCRIPTION OF PLATES.

PLATE I.

FIGS. 1 to 6. Cross-sections through various fins of *Cestracion* to show origin of concentrated mesenchyme (later giving rise to skeleton) within the fin-fold in every case. The difference in appearance between the concentrated mesenchyme in the fin-fold and the ordinary mesenchyme in the body-wall is clearly indicated. All the figures are from camera drawings.

FIG. 1. Through anterior end of pectoral fin-fold shows muscle-buds (*m*) descending into the fin. From the collection of cells below it and the partially free space above it, the bud seems to be pushing the mesenchyme cells out of its way.

FIG. 2. Through middle of same fin as Fig. 1.

FIG. 3. Through inferior caudal fin-fold. The axial skeleton is not yet evident and the mesenchyme thickening is confined to the fin-fold at this stage as it is in the other fins.

FIG. 4. Through superior caudal fin-fold.

FIG. 5. Through anterior end of first dorsal fin-fold.

FIG. 6. Through middle of the same fin shown in Fig. 5.

PLATE II.

FIG. 7. Cross-sections through pectoral girdle (*p*) and fourth gill-arch (*g*). The dotted lines connect similar points. The girdle (*p*) is in its first stage, prior to the development of the dorsal (scapular) portion, and it is still continuous with the procartilage (*b*) within the fin-fold. Note that the girdle

(*p*) is situated quite ventral to the gill-arch (*g*) and that it is also situated outside of the blood-vessels next to the ectoderm, while the gill-arch is internal to the blood-vessels and next to the enteron.

In the region indicated at "*x*" the mesenchyme cells are just beginning to take the first step in concentration toward the formation of the scapular part of the girdle.

PLATE III.

EMBRYONIC STRUCTURES OF CESTRACION.

FIGS. 8, 9, 10. A series showing the gradual approach of the pectoral girdle to the gill-arches. The fourth gill-arch is included in the drawing to show the distance between the gill-arches as compared with that between the girdle and the fifth arch. Fig. 8 shows the girdle at an early stage after the formation of its dorsal (scapular) part. Fig. 9 is a somewhat later stage (length 35 mm.). Fig. 10 is a still later stage (53 mm.). Somewhat later than this the fifth gill-arch passes partially under the girdle.

FIG. 11. The skeleton of the anal fin is differentiating out of the mesenchyme plate as a continuous anlage (*n*, neural cord; *nc*, notochord; *ca*, caudal artery, and *cv*, caudal vein).

FIGS. 12, 13. Cross-sections through the anal fin of an embryo of 40 mm. to illustrate that the fin skeleton arises independently of the axial skeleton. Fig. 12 is through the anterior end, Fig. 13 through the middle of the basale (*b*) of the fin skeleton.

FIG. 14. Cross-section showing origin of brachial artery (*b*) from dorsal aorta, and the junction of the last pair of efferent brachial arteries (*e*) with the dorsal aorta.

PLATE IV.

FIG. 15. Reconstruction of second dorsal fin of Cestracion (53 mm.) to show discrepancy of muscles (outlined in black) and skeleton (shaded) (*s*, fin spine).

FIG. 16. Camera drawing of a Van Wijhe preparation showing skeleton of anal fin of a 225 mm. embryo of Chlamydoselachus. The separation from the axial skeleton is very marked (*hs*, hæmal spines).

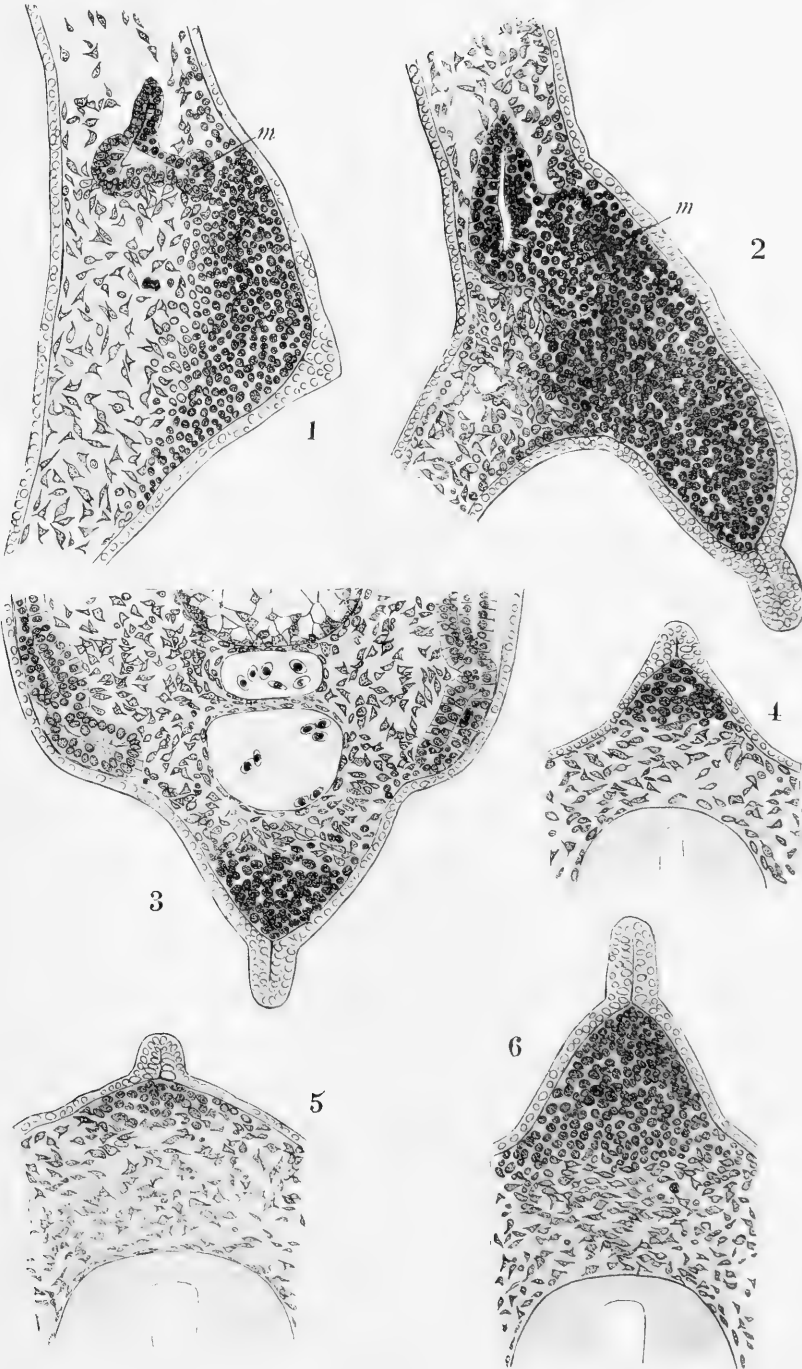
FIG. 17. Fusion of muscle-buds (*m*) in the anal fin of a 35 mm. embryo of Cestracion. Drawn with camera.

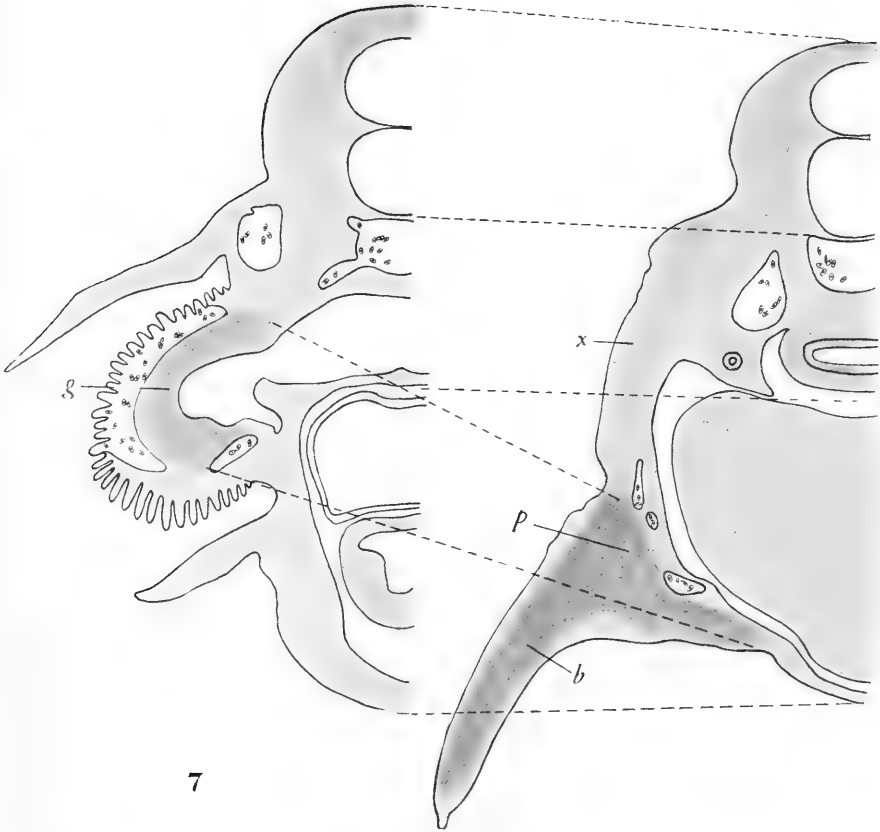
FIG. 18. Second dorsal fin of Cestracion, 58 mm., showing fusion of muscles. Camera drawing from an actual section.

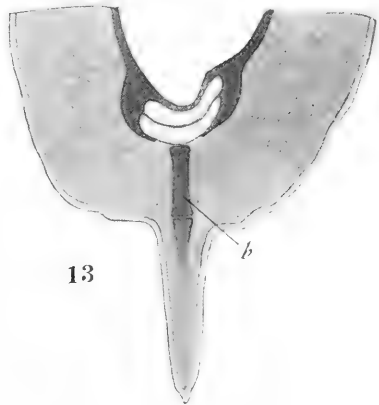
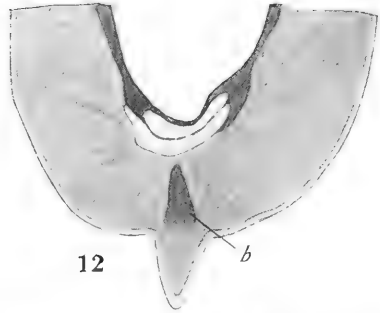
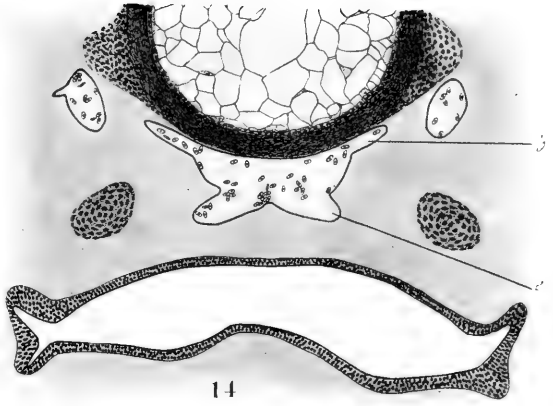
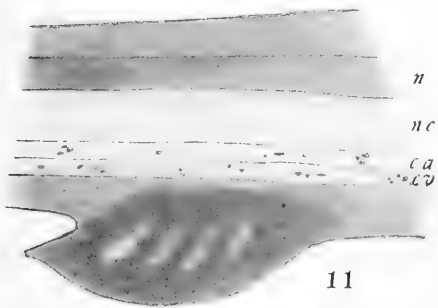
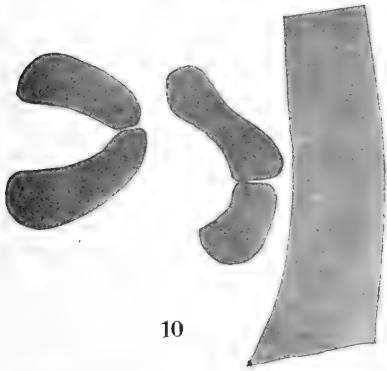
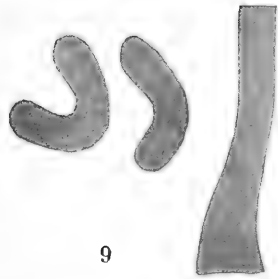
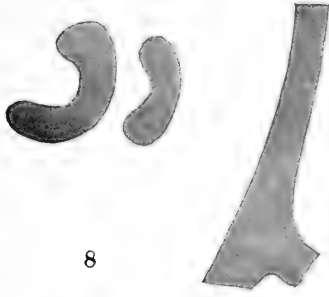
PLATE V.

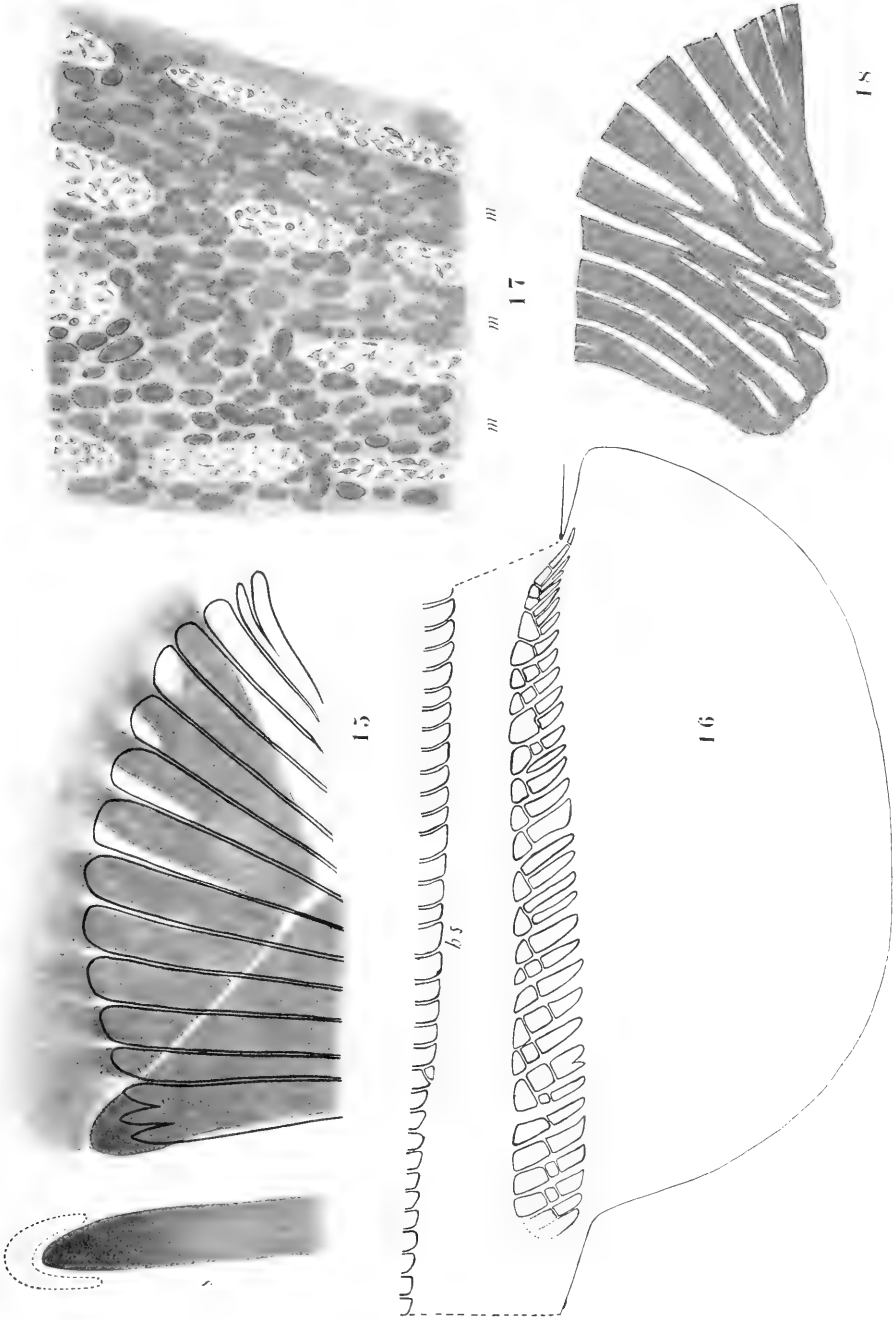
FIG. 19. Dorsal fin of Chlamydoselachus, 225 mm. Camera drawing of a Van Wijhe preparation. Note the wide separation from the axial skeleton (*sp*, spinous processes).

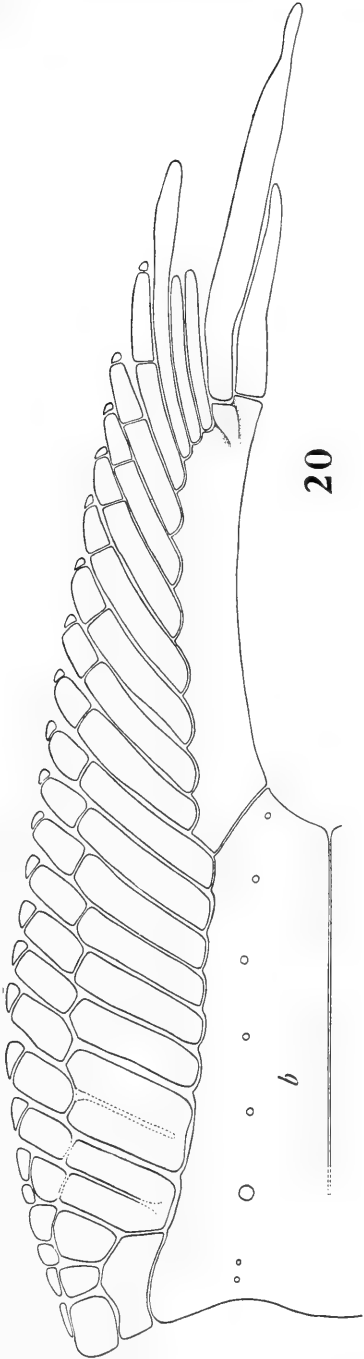
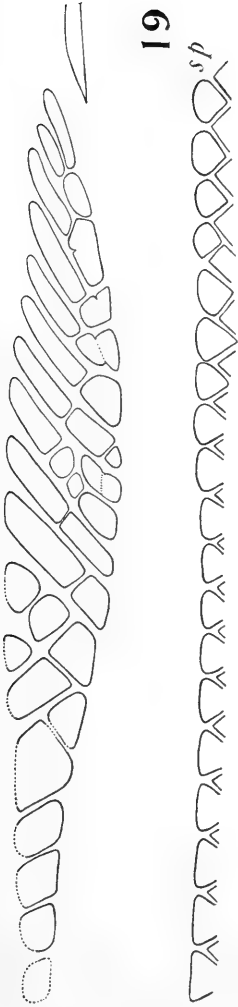
FIG. 20. Pelvic fin skeleton of Chlamydoselachus, 225 mm. Camera drawing of a Van Wijhe preparation. Note the flat girdle (*b*) pierced by eight nerve foramina, and serving as a basale for about half of the radials of the fin. The two girdles fuse at the midline in the mesenchyme stage and the separation at the anterior end is not yet complete. There is no indication as yet of the antero-median wedge-shaped element figured by Garman, 85-86, as lying between the girdles at their anterior ends in the adult.











THE BLOOD-SUPPLY OF LYMPHATIC VESSELS IN MAN.

BY

HERBERT M. EVANS.

From the Anatomical Laboratory of the Johns Hopkins University.

WITH 13 TEXT FIGURES.

Much interest attaches to the study of the vasa vasorum. Responsible for the nourishment of the vascular tree, they have acquired an added importance by the assertion, once stoutly maintained, that disease of them may initiate arteriosclerosis and phlebitis.¹

Easy steps led the anatomist to the discovery of the arterial and venous vasa vasorum; the coronaries, to be classed here, were early known and the unaided eye saw also the nourishing vessels of the ascending aorta and arch. It was only natural, then, that other portions of the arterial and venous trees should be explored for these structures.

That the lymphatic vessels of the body also possess proper blood-vessels, has, on the contrary, almost entirely escaped notice. Here also, however, it was impossible for observers to overlook the presence of vessels on the larger lymphatic ducts; but the bare knowledge that they were present there was perhaps nowhere more extended than is to be found in Cruikshank's² statement (1786): "I have injected in quadrupeds the arteries on the coats of the lymphatics and seen them ramifying very elegantly through their surface. These arteries must have their corresponding veins."

No account much more satisfactory than this was given to us until Dogiel³ in 1879 first noticed a beautiful capillary plexus about certain of the lymphatics in the rat's ear. This seemed of sufficient interest to

¹Their rôle, however, in most of the important pathological changes which affect the blood-vessels is at present not definitely known, despite the observations of Cornil, Quenu, Koester, and others.

²Cruikshank, W., "The Anatomy of the Absorbing Vessels." London, 1786.

³Dogiel, Alexander, Ueber ein die Lymphgefäße umspinnendes Netz von Blutcapillaren. Archiv für Mikroskopische Anatomie, Bd. 17, 1879-1880, p. 334. -

induce him to study in this connection the lymphatics of other subcutaneous tissue and of the mesentery. In a second paper, 1883,⁴ Dogiel noted the same "perilymphatic" plexus on the lymphatics of the capsule of the dog's kidney, but failed to see them about the lymph channels of the periosteum or gall-bladder, failures which he attributed largely to imperfect injections. His papers, written about twenty-five years ago, form apparently the sole contribution to this subject.⁵

It is interesting to note that the structures which Dogiel described were not considered vasa vasorum solely because blood-vessels of a corresponding caliber possessed none! His theory of the significance of the encircling vessels was then of necessity far-fetched. "Es ist vorläufig, unmöglich," he says, "eine stricte Antwort zu geben, aber denkbar wäre es, dass bei praller Füllung das Capillarnetz einen Druck auf das Lymphgefäss ausübt und dadurch eine Fortbewegung der Lymphe in der Richtung des geringeren Widerstandes begünstigt." Unfortunately he gives no measurement of the lymphatic vessels studied, but it is evident from his text and figures that they were rather the smaller lymphatics, though possessing muscle rings. The larger vessels, including the very largest, and the smallest ones had not been examined, nor is there any consideration of what changes occur in the type of circulation in ducts of differing caliber.

In a study of the blood-vessels of the human small intestine, I was astonished to see the blood-supply to the lacteals, easily distinguished in small spread preparations of the mesentery. The minute plexus of capillaries about these vessels was so distinct that it was easy to trace the course of the lacteal when the clearing agent had rendered its walls transparent. The investigation was merely a pleasant by-path, as it were, in the larger problem of the intestinal vessels. At the suggestion of Professor Mall, in view of the fact that it was possible to secure here an excellent demonstration of the lymphatic vasa vasorum, I have given a brief description of them as studied in the lacteals of man. The facts, however, seem of quite general significance, inasmuch as I have also examined in this connection lymphatics in the dog, the cat, and especially the sheep and goat. (In the sheep the collecting lacteals are

⁴ Dogiel, Alexander, Ueber die Beziehungen Zwischen Blut- und Lymphgefässen. Arch. f. Mik. Anat., Bd. 22, 1883, p. 608.

⁵ Since this was written my attention has been called to the mention of blood vessels supplying the pleural lymphatics by Dr. W. S. Miller in the Proceedings of the Association of American Anatomists, Twenty-Second Session, March, 1907, Anatomical Record No. 4, this Journal—Vol. VI, No. 4.

so few and consequently large, that they afford a particularly favorable opportunity to study the lymphatic vasa vasorum. One is surprised to see how profuse a blood-vascular net is closely embedded in the walls of these thin and delicate tubes.)

METHODS.

In this study only those injections were used which had been long continued at low pressure. Carmine-gelatine was employed and was made up in general, according to the method given by Walker.⁶ With such injections the capillary bed was entirely filled. In one case, ultramarine-blue was afterwards injected into the vein. The superior mesenteric trunks were invariably taken and their colic and ileo-colic branches, carefully ligated. The tissue was secured from one to five hours after death. After sufficient time in 95 per cent alcohol, the mesentery was examined in comparatively large pieces under the binocular microscope. Areas including lymphatic vessels of interest were generally cleared in creosote and mounted in toto on large glass slides in balsam or damar. It was usually necessary to carefully dissect out the larger ducts after they were cleared, for they are often surrounded by masses of fat, even in mesenteries where fat is slight. The binocular enabled the most delicate dissections to be made. Other ducts were embedded in paraffine and sectioned serially at 10 and 20 microns, to show the histology of the lacteal walls.

The lymphatics of the intestine itself were studied by stripping off the serosa with the subjacent muscularis and by dissection of the sub-mucosal tunic. All drawings were done under the camera lucida and are thus as exact representations as I could secure.

OBSERVATIONS.

It is remarkable how small a lymphatic vessel may yet have some appreciable special blood-supply. Dogiel was led to believe that the "retaining" capillary net which he had seen was present along the lymphatic vessel until the lymph capillaries were reached. I am unable to verify this statement but it will serve to call attention to the decided smallness which the lymphatic channel may reach and still be supplied by blood capillaries.

In the case of vessels somewhat larger than lymphatic capillaries, the lacteal is usually supplied by a single accompanying capillary and in

⁶Walker, G., The Blood-Vessels of the Prostate Gland. Amer. Jour. of Anat., Vol. V, No. 1, pp. 73-78, December, 1905.

vessels but slightly greater than this, with a capillary on either side of it. These accompanying capillary vessels are supplied and drained at many points by slender arterioles and venules and occasionally send a single connecting branch at right angles across the lymphatic. Such a simple scheme is figured in Fig. 1. This is frequently the only circulation possessed by lymphatic vessels until their caliber exceeds about thirteen-hundredths of a millimeter (.13 mm.) in diameter.

In the case of lacteals measuring between fifteen-hundredths (.15) and four-tenths (.4) of a millimeter in diameter it is almost always possible to find an accompanying artery and vein at least on one side of

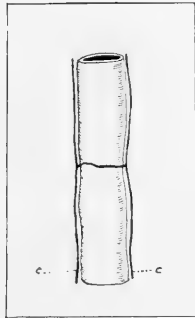


Fig. 1.

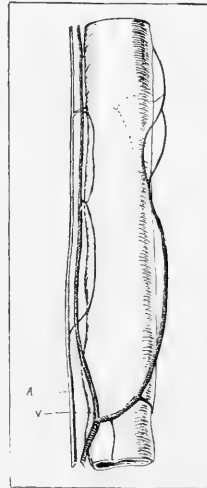


Fig. 2.

FIG. 1. Small lacteal in human mesentery, showing accompanying capillaries on either side with cross branch. $\times 50$.

FIG. 2. Human lacteal two-tenths of a millimeter in diameter. The accompanying artery and vein are seen and the capillary loops encroaching on the lymphatic wall. $\times 50$.

the lymphatic. From these vessels, slender capillary loops extend out towards the center of the lacteal wall and in most cases, extend at some place across it. Such a type of circulation is shown in Fig. 2.

In lacteals from four to five-tenths of a millimeter in diameter and above this, the accompanying vessels have often a diameter of at least 30 or 40 microns. Artery and vein, with little diminution of size, may be traced for relatively great distances, coursing with the lacteal. Often they are duplicated on the opposite side of the lacteal so that really four

accompanying vessels exist. Such a lymphatic may be said to have a bilateral blood-supply. Not only are these accompanying vessels at opposite points on the circumference of the lacteal wall, but the col-

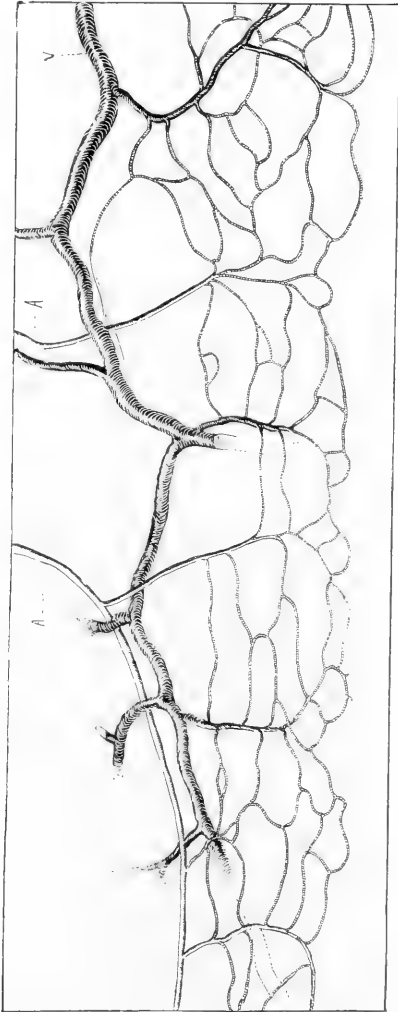


FIG. 3. The blood-supply of a large serosal lymphatic from the walls of the human ileum. $\times 45$.

lapsed lymphatic always flattens so that they may be found coursing along its lateral margins. These may then be known as axes of blood-supply and are single or double, according as the supply is unilateral or bilateral.

The lymphatics of the serosa of the intestine are particularly interesting in man since they are so large and take up relatively so great a portion of its surface. Indeed when they are flattened in collapse against the intestine, their capillaries seem to constitute a special blood-supply for the serosa and it is at first easy to mistake them for that. The supply of these serosal lymphatics is almost always by a special set of capillaries which overspreads their outer surface only. Their under

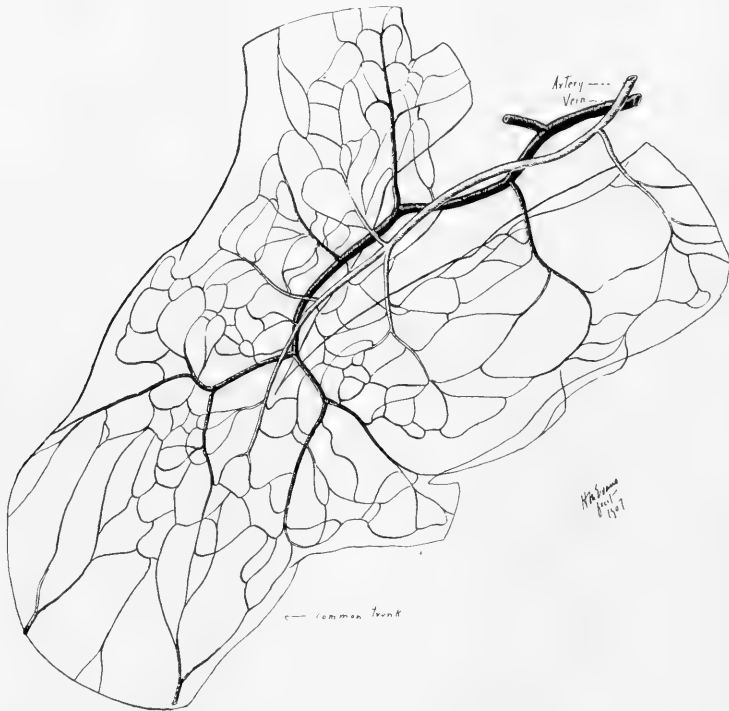


FIG. 4. Showing the blood-supply at the angle of junction of two serosal lymphatics. $\times 40$.

surface is supplied by the capillaries of the longitudinal muscle layer, with which they are in contact. Long slender arteries and veins, usually from the submucosa, penetrating the muscle coats, which they supply, reach the serosa and course there with the lymphatic channels. From these vessels, often in beautiful regularity, arteriole and venule alternately supply and drain the capillary plexus which is thrown over the lymphatic's outer surface. We may call one of these arterioles, with its corresponding

venules, a unit but the size of such a unit will be found to vary with that of the lymphatic. In some places, however, the size of these vessels is fairly constant, and their distance apart is apparently only governed by the length of the capillary bed. Fig. 3 shows such an example. The

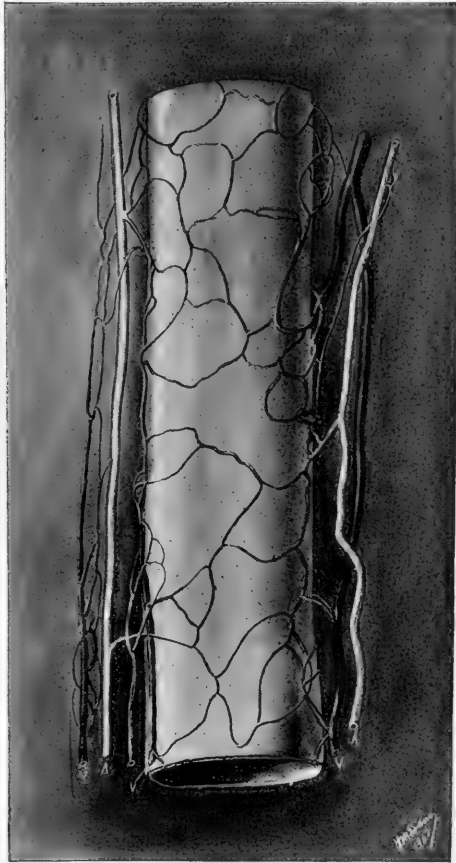


FIG. 5. Blood-supply of lacteal of a diameter of half a millimeter. Accompanying artery and vein on each side. $\times 50$.

delicate plexus formed is commonly so distinct and communicates with that of the muscularis so seldom that it is not possible to mistake it.

One may sometimes see the junction of two of these larger serosal lymphatics supplied by the tips of an artery and vein which reach the angle between the two, after having supplied both trunks for some

little distance, with a regular succession of artery and vein. Such an arrangement, diagrammatically plain, is shown in the drawing of Fig. 4.

Lacteals whose diameter is in the neighborhood of one-half a millimeter are often seen with a still further elaboration of the scheme of circulation shown in Fig. 2. The blood-supply, if bilateral, consists of a beautiful wide-meshed capillary net stretched on the two sides between the accompanying vessels. Fig. 5 shows a typical instance of such a type of circulation. It is sometimes possible to observe a perfectly regular arrangement of these tiny arterioles and venules which have to do with the capillary mesh over the lymphatic. In a very few instances, I have

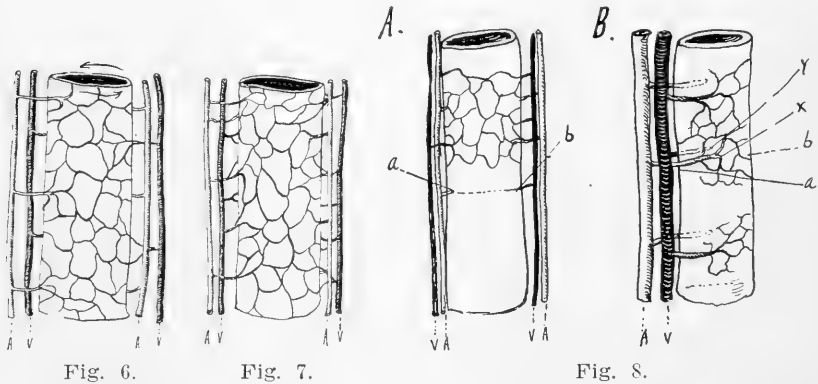


Fig. 6.

Fig. 7.

Fig. 8.

FIG. 6. Diagram illustrating supply of one-half of lacteal wall from one accompanying artery and drainage by opposite vein.

FIG. 7. Diagram illustrating supply of either side of lacteal wall from both accompanying vessels.

FIG. 8. Diagram illustrating accommodations to secure uniform capillary length in lymphatics supplied from one or two sides.

noticed that for some distance, one may find that the arterioles on one side of the lymphatic give capillaries only to one side and the venules there receive capillaries only from the other side. Also the points of origin of these small vessels from the long-accompanying stems may regularly alternate. The diagram of Fig. 6 will illustrate this. Such a type of circulation insures a separate capillary current on the two sides of the lymphatic and one which in general is in opposite directions on the two sides. The diagram shows a plan of circulation dependent on an almost exact equality of all arterial and venous stemlets. In reality such seldom occurs. Even with the irregularity caused by the occurrence now and then of larger or smaller arterioles and venules and the consequent unequal

areas of supply or drainage, the alternation of vein and artery is almost always present. On the whole, however, it is commoner to find that from either side, capillaries are given to both sides of the lymphatic. This also may occur in a fairly regular plan, approximating the condition shown in Fig. 7.

According as the blood-supply is unilateral or bilateral in character, we can observe in all lymphatic ducts certain differences in the plan of circulation which are easily correlated with the maintenance of a constant length of capillary bed. This will be readily seen by reference to the diagrams *A* and *B* in Fig. 8. In *A*, the duct is so chosen that its

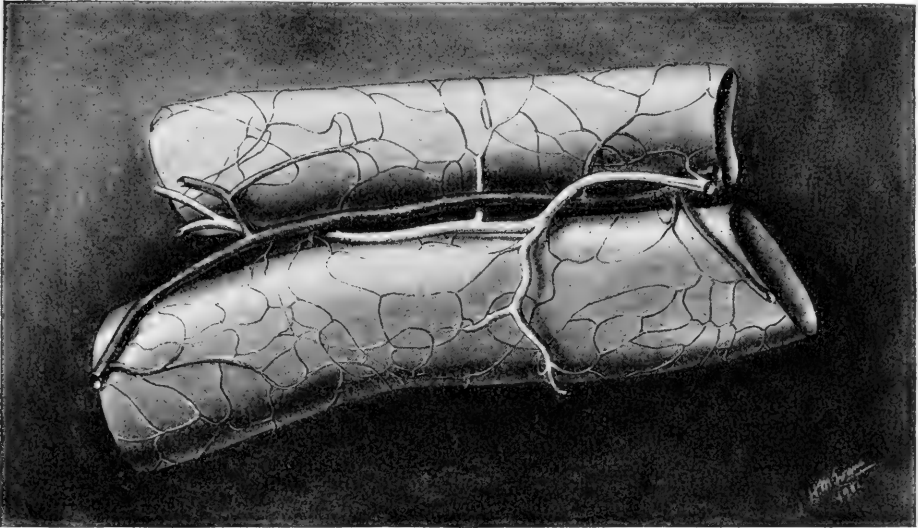


FIG. 9. Illustrating unilateral blood-supply of vessels one-half millimeter in diameter. $\times 50$.

diameter is approximately equal to the length of the capillary path, *i. e.*, from the point *a* to the point *b*. In *B*, the duct is of the same size, but as the supply is only unilateral, the existence of merely capillary paths on the lymphatic wall would necessitate the length of the capillary bed being double. Here, however, an artery of a length, half the distance from *a* to *b* extends out over the lymphatic wall to the point *x*; and a corresponding vein on the other side extends to the point *y*. The distance from *x* to *y* is thus equal to that from *a* to *b*.

Fig. 9 shows two lacteals about equal in size to that figured in Fig. 5;

but with a unilateral supply necessitating comparatively large arterial and venous vasa vasorum.

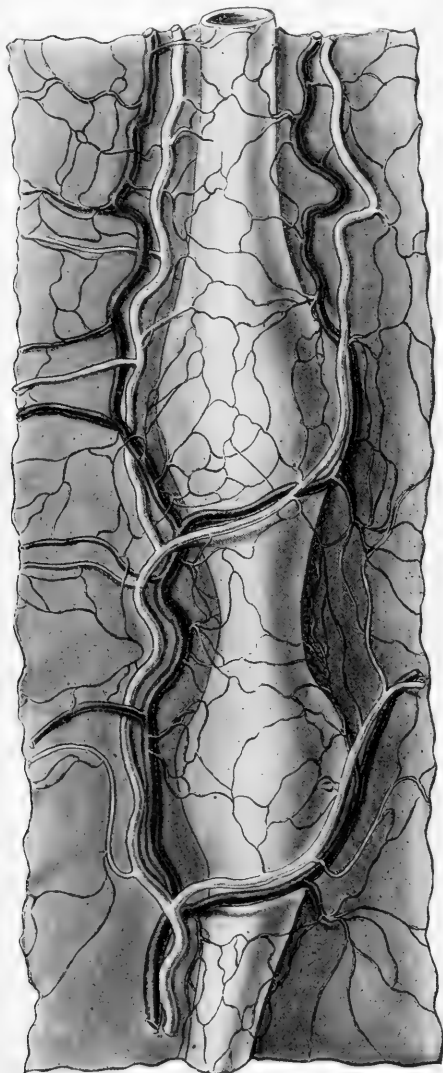


FIG. 10. Partially filled lacteal in mesentery of upper ileum, just after leaving gut wall. $\times 50$.

In lymphatics of the size we have just been considering, the capillary net is really hung between the accompanying vessels and appears so

external to the lymphatic wall that it well might suggest a "retaining net." The capillaries, however, are closely bound to the lymphatic, as will be mentioned when the structure of the lacteal wall is considered, and as can easily be seen by attempted dissection under the binocular. The net which supplies the lymphatics is never the only one which arises from the long-accompanying vessels, for these always supply the mesentery in the region where they course. This is well seen in the lacteal shown in Fig. 10, with the mesentery attached. Delamere,⁷ in speaking of the supra-valvular enlargements in the lymphatic vessels homologizes them

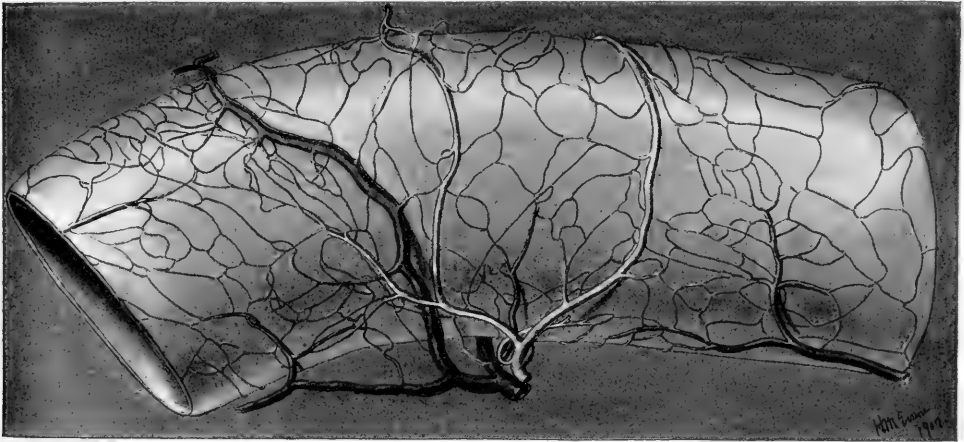


FIG. 11. Showing unilateral blood-supply of one of the larger^{*} collecting lacteals. $\times 50$.

with the true contractile sacs in some vertebrates. No very noticeable increase of muscle tissue can always be demonstrated here, nor is there usually much increase in the closeness of the vascular net supplying these areas. Fig. 10 gives the common appearance of the vasa vasorum in the neighborhood of the valves. It will be noticed that the long-accompanying vessels send strong cross branches just below the valves. This is by no means an invariable plan but I have found that, on the whole, such cross branches are commoner here than elsewhere.

The larger collecting lymphatics of the human mesentery show little or no change in the essential plan of blood-supply. Here, too, accompany-

⁷ Poirier and Charpey, "A Treatise of Human Anatomy"—The Lymphatics—General Anatomy, by G. Delamere. English Ed. Trans. by C. H. Leaf, Chicago, 1904.

ing vessels exist and the supply is unilateral or bilateral. The consequent picture is merely an elaboration of that already described. Vessels

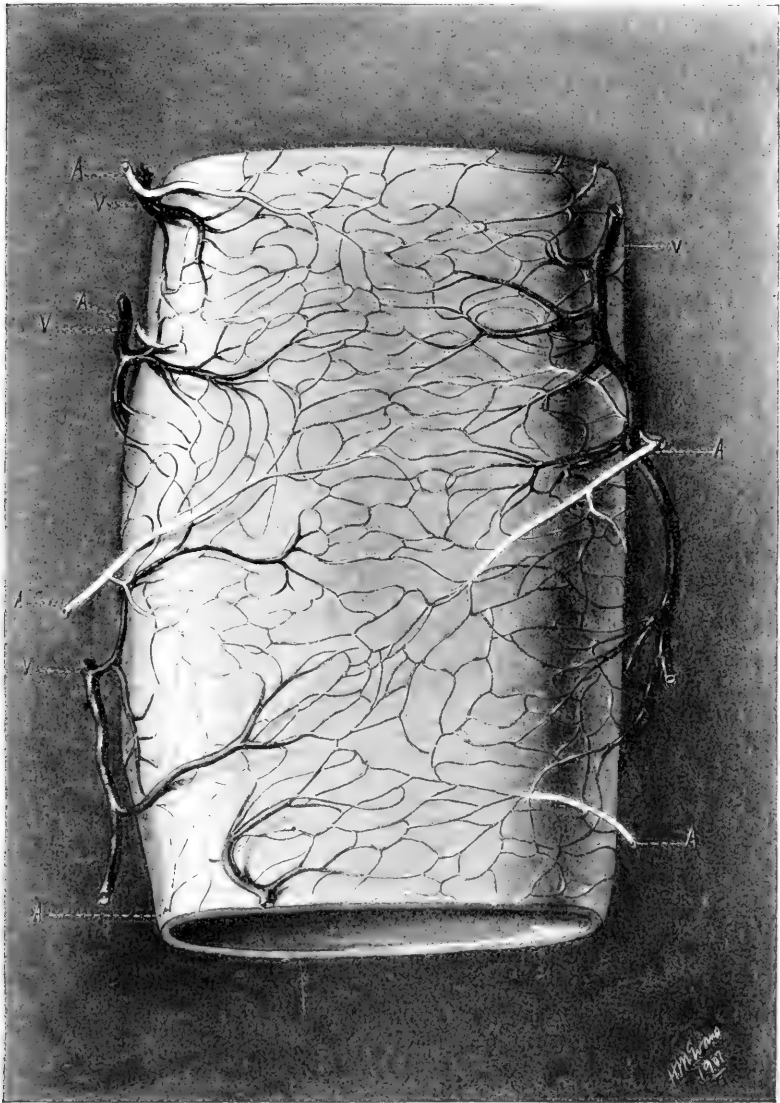


FIG. 12. Showing bilateral blood-supply of large collecting lacteal, near superior mesenteric trunks. $\times 50$.

with a unilateral supply have relatively large arteries and veins on their walls. Fig. 11 gives such an example. The capillary mesh is always

closer in these vessels than in the smaller ones, a fact which seems in accord with the greater thickness of the lymphatic walls. It is in vessels similar to that shown in this figure that one may find sometimes complete arterial rings from which capillaries are given off.



FIG. 13. Cross-section of lacteal from the mesentery of the lower jejunum of man. $\times 100$. *c* = Capillaries, vasa vasorum.

Fig. 12 shows one of the largest ducts near the superior mesenteric trunks. The supply is bilateral and one sees here also an alternation of arterial and venous stems among the vasa vasorum. It is significant that the plan involves merely an increase of that shown in Figs. 2 and 5. There is seldom any tendency to form more than this one plexus in the

lacteal wall, though in these largest vessels, in places, capillary loops and meshes are seen superficial to the plane of the chief plexus. These supply the outermost connective tissue and fat, but appear never to form another well-defined layer of capillaries such as exists in the sheaths of the larger blood-vessels.

It will be well here to refer to the actual place of the vasa vasorum in the lymphatic wall. Seen in section these are always adventitial in position. Occasionally a capillary twig underlies a few muscle fibers, but it is even then in a small septum of connective tissue, as shown by a Van Gieson stain. Such stains show clearly the remarkable proportion of the lacteal wall taken by the muscular media. Fig. 13 shows a typical section of these ducts in man. From within outward one notices the characteristic projection of the endothelial nuclei into the lumen, the slight subendothelial tissue (barely demonstrable), the strong media, the vasa vasorum, and finally, the connective tissue and fat of the adventitia. The absence of much subendothelial tissue and the presence of so strong a media, are probably characters peculiar to the human lacteals, but the adventitial position of the lymphatic vasa vasorum seems a general fact. In this respect, then, the lymphatic trunks resemble the arteries rather than the veins, whose media is copiously supplied with nourishing vessels.

The chief facts brought forward in this paper may briefly be summed up as follows:

1. Lymphatic vessels possess a special blood-supply when possessing a caliber far below that of blood-vessels which have vasa vasorum.

2. The lymphatic vessel is typically supplied along one or two axes by long-accompanying arteries and veins which give off a capillary plexus.

3. This capillary plexus is truly adventitial in character but rests directly on the muscular media, in this respect resembling that of the arterial walls.

4. The plan of circulation is essentially the same in all ducts above a medium caliber, though the capillary mesh is closer in the larger vessels.

5. Whether the supply is unilateral or bilateral, an accommodation in the extent and size of the arterial and venous vasa vasorum is made in accordance with a capillary bed of uniform length.

It is a pleasant duty to express here my thanks to Professor Mall. I must acknowledge, also, the very kind assistance of Mr. Broedel, under whom a portion of the drawings were made.

NOTES ON ACANTHODIAN SHARKS.

BY

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From the Zoölogical Laboratory of Columbia University.

WITH 36 FIGURES.

The following notes relating to Acanthodian material preserved in several European museums were brought together several years ago as an incident to another study, but they have now been transcribed for publication, since they deal with several unnoted, or little noted, features of these ancient sharks.

The Acanthodians are known to have constituted the earliest line of sharks which underwent a period of evolutionary prosperity. First known from the late Silurian, they gave rise to a series of highly modified forms in the Devonian (represented by at least three families and upward of twenty species), and became extinct by the close of the Permian. The earliest of the remaining groups of sharks, on the other hand, do not appear before the Devonian and were indeed sparingly represented during the maximum epoch of the Acanthodian; but by the coal times they had evolved characters which, as we have reason to believe, placed them under more favorable conditions for survival and thus enabled them to supersede their more specialized Acanthodian neighbors.

Dentition.—The dental structures of Acanthodians have received but little comment. It was known that while in many genera, Acanthodes, Parexus, Diplacanthus, Climatius, and Cheiracanthus, teeth are rudimentary (or even lacking), in other forms they are exceedingly conspicuous. Thus, in Acanthodopsis, according to A. S. Woodward, there are “a few large, laterally compressed, triangular teeth”; in Ischnacanthus, “a few large conical teeth, the interspaces between these teeth being occupied by a close series of minute cusps, all apparently in firm connection with a membrane bone in both jaws.” But no details of dentition have been given, in spite of the fact that they are of no little importance in comparing Acanthodians with other early sharks. Accordingly, we are led to give the accompanying figures, 1-11, with comments.

There are present in *Ischnacanthus gracilis*, cf. Fig. 1 (from specimen

in the Edinburgh Collection), teeth arranged in a graded series with stouter ones at the side and with longer and narrower ones in front (similar, accordingly, to the conditions in Cladoselache and many modern sharks); the spaces between the teeth, however, appear somewhat irregular (artifact?). In Fig. 2, in a similar specimen, the heavier teeth show lateral cusps, distinctly cladodont in type. On each side of the jaw there can be counted about a dozen marginal teeth. There is also shown in this specimen (noted for the first time in Acanthodians) a second or successional row of teeth, *suc*; these are distinctly smaller than the functional teeth, and cannot be mistaken for the elements of the opposite half of the mandible. In Fig. 3 (British Museum, P. 6996) is shown a mandible of *Ischnacanthus* which illustrates strikingly the shape and prominence of the lateral teeth. In Fig. 4 (Edinburgh) there appear again the prominent lateral teeth, but with indications that their bases were somewhat as in other sharks,—not fused (as Smith Woodward and others believed) with the “membranous calcifications” of both jaws. The separateness of the teeth is also indicated in similar specimens (*I. gracilis*, in Edinburgh), shown in Figs. 5-8; in the first of these the bases of the separate teeth are particularly well shown, including the interlap of the bases, which here occurs very much after the fashion of certain modern sharks.¹ Interesting, also, is the arrangement of minor cusps on either side of the major one, which suggests clearly the teeth of Cladodonts. Strikingly Cladodont, moreover, are the various forms of teeth occurring in different regions of the mouth. Thus, the form of tooth shown in Fig. 9 occurs more nearly symphyseal than the form in Fig. 6, and this in turn precedes that of Fig. 7; in Fig. 8 is a detail of a favorably preserved series of lateral teeth. In the matter of the successional series of teeth no more definite result could be obtained than that shown in Fig. 2; *i. e.*, indicating a replacement of teeth, and showing in a more marked way than in Cladodonts or in modern sharks, a difference in size between the old and the new teeth.² The growth of the new teeth, we therefore infer, was conditioned quite differently than in the dental fold of later sharks. A single specimen (Edinburgh), Fig. 10, confirms the foregoing account of dental succession; it preserves, it is

¹ Cf. especially Figs. 6 and 8.

² There is accordingly a suggestion that in this order of sharks a dental trench and a successional series of teeth had not been perfectly attained; in the event of the loss of its large teeth, such an Acanthodian as *I. gracilis* would evidently have to depend upon the small teeth in the second row. That these teeth could then increase in size and finally equal the large teeth remains an open question; but it is clear that teeth could not be replaced as readily as in a modern shark.

true, only a fragment of the jaw, showing in visceral aspect the region of its articular margin. Here it presents a series of enlarged denticles which pass from the condition of shagreen tubercles into the marginal teeth; but it will be seen that as the submarginal denticles pass forward and outward toward the rim of the jaw, they become reduced to a single successional row, as already noted in Fig. 2.

In the genus *Acanthodopsis* a remarkably perfect dentition is shown in a specimen in the British Museum (Fig. 11). In each ramus there was present a row of a dozen (possibly there were several more) teeth which increase in size as they pass toward the region of the symphysis. They are certainly stouter in this form than in *Ischnacanthus*, and the symphyseal teeth were as conspicuous, or even more conspicuous, than the similarly situated teeth in a modern *Odontaspid* shark. Moreover, that they were here functionally important seems evident from the thickness of the anterior reach of the meckelian cartilage.

From the evidence of dentition, accordingly, *Acanthodians*, in certain of the genera at least, resemble Devonian *Cladodonts*, and from this structural standpoint, there is suggested a closer kinship between the groups.³

³ That the *Acanthodians* were in a general sense planktonophagous, as Dollo has recently suggested, '06, on the ground that in several genera teeth have not been described, is by no means evident. It is certainly possible, in view of the evidence that a widely diversified evolution took place within this group, that some of its members were specialized to secure plankton, just as were members of other optimum groups, euselachians, ganoids, teleosts. It is, however, clearly safer to conclude, as has hitherto been done, that the *Acanthodians* in which the dentition was reduced (or rudimentary) were forms whose diet was restricted to small or minute organisms. To specify that this food was plankton, and that the structures of the fish were typically planktonophilous, carries one beyond the limits of evidence. Nor does it seem safe to assume, as Dollo has done, that those *Acanthodians* which were not of this form were developed with an abyssal type of dentition, after the fashion of *Stomias*, *Aulops*, etc., seems equally wide of the mark. The present notes indicate a rather normal type of selachian dentition, primitive, we infer, in as much as the mouth-invading shagreen seems to have gone no farther than the mouth rim,—there being no evidence of a wide successional series of teeth as in euselachii. And we can assume, accordingly, that in the *Acanthodian*, as in other fishes, *Characinids* for example, large teeth predicate large prey and rapacious habits. But not necessarily bathybial. There is evidence on the other hand that *Acanthodians* could not have inhabited deep water, for the rule is fixed that bathybial forms, on account of the mechanical conditions of their habitat, are extremely defective in hard structures, scales, bones, and spines. The stout-walled spines and dense shagreen of *Acanthodians* should alone have precluded the assumption that they were deep-water forms.

Skull and Branchial Arches.—To discuss the complicated structure of the skull and branchial arches of Acanthodians is hardly the part of the present paper. I have had, however, thanks to the courtesy of Professor Jaekel, the privilege of examining the extraordinary and beautifully prepared series of the Permian species, *A. bronni*, in the Berlin Museum. And I am able to confirm the essential details given for this species, *i. e.*, the subdivision of the pterygo-quadrate and meckelian cartilages, as shown in Jaekel's Fig. 1, in *Zeitschr. deutsch. geolog. Gesell.*, 99, or Reis's Fig. 1, *Schwalbe's Morph. Arbeiten*, Vol. VI.⁴ One certainly finds little reason to dissent from the interpretation of these elements in terms of the succeeding branchial arches, for, in view for example of the conditions in the young Chimaeroid, we have clearly the grounds for concluding that the mandibular arch of primitive elasmobranchs was probably a structure segmented like a branchial arch. Thus, in *Acanthodes bronni* (Fig. 12), the epibranchial element is represented in the mandibular arch by the element *q*, the pharyngo-branchial by *pt*, the cerato-branchial by *m*, the basi-branchial by *m'*, and the hypo-branchial by *c*. What the anterior element, *a*, represents is by no means as probable. I am not convinced that Jaekel's conclusion is a just one in regarding this as the "primary maxilla," nor indeed his transcendental views as to the homologies of such parts of the mandibular arch as an "infra dental," or an "articular," in his endeavor to demonstrate homologies between subdivisions of the Acanthodian mandible and the dermal bones of the jaw of higher forms.⁵ As regards the dermal sub-meckelian element, *m''*, one can also hardly subscribe unreservedly to the view that it represents the definite splenial of higher forms, for there is the obvious possibility, in view always of the fact that there are here no other dermal bones associated with the mandibular arch, that this element may be *sui generis*, without, however, going to the extreme view of Reis that it functioned as an extra-mandibular spine, which churned up the bottom and aided in securing the hidden food of the fish.

This view of Reis, it may be added parenthetically, is founded in part upon his assumption that this *dermal* sub-meckelian element bore a series of appendages somewhat as branchiostegal rays. I have, however, a suspicion—for a final judgment one should of course compare Reis's material—that the present appendages belonged in reality to the underlying hyoidean arch; they are beautifully shown, for example, in the

⁴ Cf. the present Fig. 12.

⁵ *SB. Gesell. naturforsch. Fr. Berlin*, 1905, p. 134 *et seq.*

Berlin specimen from which Fig. 12 was prepared. In any event they indicate that the ceratohyal region was more perfectly gill-bearing than in modern sharks.⁶

That the mandibular arch in *Acanthodes bronni* shows segmentation must, accordingly, be admitted; and that its elements indicate homologies with the branchial arches. On the other hand, unfortunately, these homologies cannot be accepted as final, for the following reason: The condition of the Permian species, *A. bronni* is not confirmed by the condition in several of the Lower Devonian forms. I refer particularly to such a specimen, for example, as that of *Ischnacanthus gracilis*⁷ (Fig. 13), in which no separate elements can be distinguished; and this is true, also, in a well-preserved jaw arch of *Cheiracanthus murichisoni* (Edinburgh). For it is obvious that if the earlier Acanthodians show no trace of these elements, the condition in the much later forms may, like Cope's "elements" in the crania of Xenacanth, be interpreted as artifact.

As far as the writer is aware the roofing of the Acanthodian skull with dermal elements has never been described in detail. Its interest, however, is patent, in as much as it represents an early, if not the earliest, form of strengthening the brain capsule in the gnathostome series. In certain genera it undoubtedly forms an effective shield for the brain, although, morphologically speaking, it must be regarded as but a parallelism of the dermal head shield of the higher fishes. For it consists not of a series of plates, each formed of fused shagreen elements, as one knows it in the ontogeny of recent fishes, but of a series of single, although greatly enlarged shagreen elements. Thus we see, for example, in *Climatius scutigera*⁸ (Fig. 14), that the broad head roof is protected with dermal plates, numerous (a score or more), arranged irregularly, not closely opposed to one another, and clearly not to be regarded as the homologues of parietals, frontals, pre- and postorbitals, etc. From their shape and radial ornamentation, they are obviously to be compared with the enlarged dermal denticles of many other elasmobranchs, *e. g.*, rays.⁹ It is evident, furthermore, from a comparison of the head-roofing

⁶ Dollo's recent suggestion, '06, that Acanthodians are plankton-eating forms, is recalled by these laminae—if they be interpreted as branchiostegal, and not as traces of gill filaments.

⁷ Powrie Collection, No. 258, Edinburgh.

⁸ Brit. Mus. 35,908.

⁹ Cf. in this regard (Fig. 15) an enlarged dermal denticle from the head-roof of *Climatius reticulatus* in the Edinburgh Museum.

plates with the other dermal defenses in Acanthodians, that there exists an evolutionary range in the shape, size, and character of these structures; from the small denticles of the trunk of the fish we may trace transitional forms to the enlarged denticles which margin the structures of the lateral line, to those which constitute the dermal head-roof, and finally, to those which margin the eye; the last sometimes seven or eight in number, instead of the four usually described. That the dermal denticles of the trunk may in themselves be subject to an evolutionary range of forms is also evident; in certain species and genera these denticles may acquire the quadrate margins (Figs. 33 and 34), which suggest the bony plates of ganoids (fishes with which Agassiz and others associated them), others, on the contrary (Fig. 36), may imbricate their margins to such a degree as to suggest superficially the scales of teleosts. Moreover, prominent contour lines may be defended with rows of enlarged dermal denticles (already noted by Smith Woodward) to such a degree as to suggest the analogous scales of sturgeons or siluroids. Thus, in the specimen (*Climatius scutigera*), pictured in Fig. 14, enlarged denticles, shown at *r s*, form a ridge between the head and the dorsal fin. In such a ridge as many as ten successive elements may be counted.¹⁰

A final word regarding the head-roofing denticles. In some forms they may be small and tubercular, in others large and tubercular;¹¹ in still other forms they may be flattened and closely compacted.¹²

Vertebral Column.—The vertebral axis of Acanthodians is known to retain its notochordal condition and the arrangement of neural and hæmal spines has already been figured by several authors, Reis notably. Specimens, however, are rare in which these axial relations may be determined, and I might refer in this regard to a specimen of *Ischnacanthus gracilis*¹³ (Fig. 16). Here the neural and hæmal arches appear as distinct (metamerical?) elements; as in *Cladoselachus* there are no interneurals, and there is no evidence that the arches approached closely to the surface of the body (*i. e.*, are not connected with the bases of fins). A remarkable condition, on the other hand, is shown in a specimen of *Ischnacanthus gracilis* (Fig. 17), (counterpart of Powrie's type, No. 251, Edinburgh Collection), in which appears a series of superficial elements, *b*, in front

¹⁰ Specimens in Brit. Mus., P. 6955-56.

¹¹ Cf. specimens of *Parexus recurvus* and *falcatus*, in Edinburgh.

¹² Cf. *Euthacanthus macnicoli*, Edinburgh.

¹³ No. 334 in the Edinburgh collection.

of and in the neighborhood of the dorsal fins. They are distinctly separate from the neural arches and are apparently metameral; their interest is obvious from the standpoint of fin morphology, for they can be interpreted as the rudiments of the basalia of a more continuous type of unpaired fin.

Fins and Girdles.—The fins of Acanthodians are beyond peradventure of a lateral fin-fold type, and as such they have been given a prominent place in the much-discussed problem of the origin of the vertebrate limbs. From this standpoint it is clear, either that the limb structure of Acanthodians and other primitive sharks must be reducible to a common plan, or that the curious spine supported Acanthodian webs must have arisen *sui generis*. The latter view is difficult to accept, since it is conceded unanimously that the paired limbs of all other vertebrates are homologous, and it seems, therefore, if only from purely *à priori* grounds, illogical to assume that Acanthodians, which are sharks in so many details of structure, could not have had fins based essentially upon shark-like structures. The view, moreover, of the homology of the fins of Acanthodians and sharks¹⁴ is supported by the evidence of Cladoselachian sharks whose fin structures are in important regards intermediate in type. In both forms the paired fins functioned as balancing organs, rather than as paddles, and in Cladoselachians there is a concentration of the supporting elements, radials, in the anterior rim of the fin which, I have maintained, served as the ancestral condition of the spine of the Acanthodian.¹⁵ This view is supported by the following facts: The caudal fin of Acanthodians shows the radials in the process of concentration in the anterior rim of the fin. There is no spine present, although the anterior fin margin is encrusted and stiffened by shagreen. Such a condition has already been noted by various writers; it is admirably seen in one of Professor Jaekel's beautifully prepared specimens of *Acanthodes bronni*, which I have figured herewith (Fig. 25). And similar conditions are known in other genera. In *Parexus falcatus*,¹⁶ for example, the hypural lobe of the caudal fin is strengthened by shagreen to such a degree as to

¹⁴ Cf. 1894, Jour. Morph., Vol. IX, pp. 98-111; 1896, Anat. Anz., Vol. XI, pp. 677-679; 1896, Nat. Science, Vol. VIII, pp. 245-253.

¹⁵ In this evolution the dermal elements played an important part, encrusting and strengthening the anterior rim of the fin, a process which caused or was accompanied by a reduction in the radials.

¹⁶ British Museum, P. 130.

suggest a spine-like fin support (Fig. 20). Indeed, in such a form as *Diplacanthus tenuistriatus*¹⁷ (Fig. 22) such a condition is actually attained. And the dermal elements arrange themselves in the series of rows characteristic of Acanthodian spines, while the hinder radials become obsolescent. If, accordingly, such a condition be compared with that shown in the second dorsal fin of *Euthacanthus macnicoli*¹⁸ (Fig. 23), we cannot fail to note the close correspondence; the fin spine is made up of radial components in which dermal tubercles are concentrated, and these even appear *behind* the fin spine strengthening a series of delicate rays which are evidently comparable with the obsolescent rays shown in the caudal fin of Fig. 22. A second dorsal spine of an Acanthodian is thus the homologue of the concentrated radials in a caudal fin. And it is easy to see how such a condition would readily become highly specialized in forms in which dermal structures present a wide range in their scheme of evolution. In such a broad spine, for example, as that shown in Fig. 26, *Climatius reticulatus*,¹⁹ the broad striation in its dermal crust still testifies to the component radial elements,—the basal elements, or at least some of them, forming the region *b*. In Fig. 24, *Climatius uncinatus*, a more highly modified type, there can still be seen traces of the component radials in a spine which has become curved. In other spines (Fig. 19) the dermal elements have completely obscured the core of radial elements over which primitively they have been laid down.

Admitting, then, that the fin spines of Acanthodians are founded upon the concentrated radials of a Cladoselachian fin, the puzzle of the paired fins of the Acanthodians becomes greatly simplified. And for a further study of the matter, I believe that an important hint is given us in the behavior of the dermal elements as contributing to the formation of fin spines. For if dermal elements play so important a part in strengthening the outer supports of fins, may they not also have pressed deeply into the integument and strengthened the basals? For it is these elements which have contributed no little confusion in the studies of the Acanthodian paired limbs. Without attempting to reconcile the differences which occur in the plan of the shoulder girdle and spines of various Acanthodians, I may point out that there is evidence not merely of a pair

¹⁷ Edinburgh Museum.

¹⁸ Brit. Mus., P. 35,907.

¹⁹ Brit. Mus., P. 1343.

of "inter-clavicular" or "claviculoid" membrane bones in certain genera, but that the basal region of a fin may be strengthened with *many* dermal elements. This is well shown in a specimen of *Parexus falcatus* in the British Museum, P. 130 (Fig. 21), of which an enlarged figure of the base of a pectoral fin is shown in the present Fig. 27; here as many as half a dozen dermal elements may be counted along the mesial margin of the basal plate, and it is even possible that the entire flat distal surface of this plate was strengthened by dermal elements. Accordingly, the shoulder girdle of Acanthodians, like the girdle of a teleost (siluroid, for example) is to be treated as including a dermal complex. Our present knowledge does not warrant us in attempting to homologize its elements with those in the higher forms (as, nevertheless, Jaekel has done in identifying "suprascapular," "scapular," "coracoid," and "cleithral" elements), since such structures can only be compared with the conditions in the higher vertebrates when it has been shown that the Acanthodians have closer phyletic relationships to the higher forms. Until this can be demonstrated the structural elements of the Acanthodians must evidently be interpreted in terms of their nearest kindred; *i. e.*, fossil and recent sharks. The safest conclusions in our present stage of knowledge are, I believe, these: That we are to regard the proximal portion of the shoulder girdle, the region of *sg* in Figs. 18, 19, 21 (elements *a, b, c*, of Jaekel) as equivalent to the proximal portion of the shoulder girdle in sharks; the distal portion becomes a region of concentrated fin-supporting elements, fused basals, a portion of the basal parts of the radials, with as an important if not a maximum component a dermal complex, *e. g.*, the region *d* in Fig. 19 or the more discrete elements in Fig. 27. With regard to the homology of the cartilaginous pieces which Jaekel and Reis describe in *A. bronni*, as fin-web-supporting elements placed immediately behind the pectoral spine, one may, I conclude, regard them as the remains of the series of baso-radial elements. The elements of this series had in general, as has been noted, fused at the base of the pectoral fin spine; but it is by no means improbable that in certain forms a number of the posterior elements remained discrete, retaining more or less accurately their primitive function. That there is the greatest range in the degree of concentration of fin-supporting structures in the Acanthodians all will, I think, agree who have examined these structures in many genera.

The supports of the ventral fins, so important in the general discussion of the origin of the paired limbs, have, as far as I am aware, never been described. Interesting, accordingly, is the specimen of *Diplacanthus*

striatus in the British Museum, P. 36,582, cf. P. 1757, *a* (Fig. 19), which shows a distinct pelvic girdle, *p*. At the base of the fin spine the enlarged supporting mass, *r + b*, represents, we infer, the fused basoradial elements. At *d* appears a pair of distinct plates, dermal apparently in origin. Whether they are related to the much discussed series of "fin spines passing between the pectoral and pelvic fins" is not clear.

Sense Organs.—Our knowledge of the end organs of Acanthodians appears to be confined to a few notes on the lateral line and on the large eyes protected by "four dermal plates." As to the former structure, Smith Woodward remarks²⁰ that "a single lateral line occurs high on each flank, marked not by any tubular or other excavation of the scales, but by the ridge-like displacement of two series, between which the organ originally extended. The supposed evidence of additional sensory canals appears to the present writer to be due to a misinterpretation of the displaced dorsal and ventral ridges, which exhibit no median series of scales." Reis, in this connection,²¹ has made the interesting observation that the lateral lines are joined by an occipital commissure very much as in recent elasmobranchs and teleostomes; and he refers also to the presence of a median canal in the forehead which he compares to (or parallels with?) the rostro-median canal of *Chimæra*; he figures, finally, a supraorbital canal and refers to the absence of a suborbital branch arising from the main canal in that region. Reis's figure representing these conditions²² is of especial importance. To the foregoing notes upon sensory structures I may add: The "additional sensory canals," in spite of the skeptical comment of Smith Woodward, are beautifully seen in several specimens in the British Museum and in the Edinburgh Collection. I refer particularly to *Climatius grandis* and *Ischnacanthus gracilis*²³ (Figs. 30, 31, Edinburgh, and 32, this after No. 241, Powrie Collection). In these particular forms the branching of the lateral line, *ll*, in the neighborhood of the dorsal spine is, indeed, so common an occurrence that one might even maintain that it was the normal condition. It has not, however, been observed to occur on both sides in the same individual; nor is it definite that it occurs always on the same side, although a close examination has convinced me that it occurs on the right side in most cases. The most remarkably preserved sensory structures in Acanthodians are probably shown in the specimens of *A. bronni* (from

²⁰ Cat. Foss. Fishes, Vol. II, p. 5.

²¹ Morph. Arb. Schwalbe, VI, pp. 195-196.

²² *Op. cit.*, p. 195.

²³ London, P. 6974.

Lebach) in the Berlin Museum, for the inspection of which I am greatly indebted to Professor Jaekel. For they show not only the lateral line but its many branches, the sensory canals of the head, and, most interesting of all, the auditory organs and the rim of the nasal capsule. Thus, in the specimen shown in Fig. 28, one readily distinguishes²⁴ the canals of right and left sides, *ll*, from which a series of vertical branches arise very much as in recent selachians, cf. especially in the young specimens (20-30 cm.) of *Chlamydoselachus*; the lateral lines apparently draw together in the occiput and from this region pass forward, giving off various branches, a supraorbital canal, *supra o*; there, also, appear a suborbital canal, *sub o*, an auditory capsule, *au*, of extraordinary size, and traces of sensory canals, *br s*, in the branchial region, again a condition which suggests the modern shark (*Chlamydoselachus*). These conditions are shown to even better advantage in a specimen from the same collection shown in Fig. 29. Especially noteworthy are here: (I) The position of the suborbital canal, for it is seen to arise (contrast Reis's note given above) from the main canal passing to the trunk, *i. e.*, in the position usual in fishes. (II) The great size of the sensory branches passing to the region of the gills. (III) The structures of the auditory organ, the utriculus, *utr*, and, prominently shown, a vertical simicircular canal, *vsc*. (IV) The upper margin of the nasal capsule, *nas*, which indicates accurately the position of this organ.

In the manner of the protection of the lateral line the Acanthodians show a range of evolutionary characters; in *Euthacanthus elegans* the canal is marked only by a ridge-like prominence of marginal scales (Figs. 34 and 34a); in *Acanthodes bronni* the marginal scales become enlarged and prosalient (Fig. 35); in *Euthacanthus gracilis* they may completely overlap and enclose the sensory canal (Fig. 33). It is finally observed that a great range in the manner of protecting the sensory structures is found at different regions in the same individual.

Relationship of the Acanthodians.—It has been pointed out in the foregoing notes that the Acanthodians agree in a number of regards with the Cladoselachian sharks, and that from this evidence we conclude that they are more closely related to these forms than has hitherto been generally accepted. Dental characters, structures of fins caudal, unpaired and paired, vertebral axis, even the mode of protection of the eye, are

²⁴ In *A. bronni* the shagreen denticles are reduced in size; those only are large which protect the sensory canals, hence the clearness with which the arrangement of the canals may be followed.

distinctly cladoselachian in type. Taxonomically, therefore, Cladoselachians and Acanthodians should be more closely associated than should, for example, Cladoselachians and Xenacanth, or Acanthodians and Xenacanth. We are thus led to suggest again²⁵ that a group such as defined by Pleuropterygii (sharks with fins of fin-fold type), would justly include both Cladoselachians and Acanthodians. This group, however, might more accurately be regarded as of superordinal rather than of ordinal rank, and ordinal rank would thus remain in the groups Cladoselachia and Acanthodia.

The question as to which groups, Acanthodian or Cladoselachid has retained the more primitive character is indicated in the accompanying table (v, p. 221). In the great majority of structures one can only conclude that the Acanthodians have passed through a stage in evolution which is best represented by the Cladoselachian. In one regard only (apart from the matter of size) does the latter appear the more specialized, *i. e.*, in the unsegmented character of the mandibular arch (*v*, however, *supra*).

If these things be true, it may next be queried why is it that the more specialized group is known from an earlier horizon? For certainly the appearance of the Acanthodians in the upper Silurian (as against the Cladoselachian in the upper Devonian) is in general evidence of the greater primitiveness of their group. And this is in truth a question which can be answered only by the time-worn appeal to the defectiveness of the palæontological record, noting especially in this regard that the soft structures of the Cladoselachians would be less apt to be preserved than the hard structures of the Acanthodians. We may, however, safely predict that from the earliest Acanthodian horizon there will be discovered forms which will represent the ancestors of all of the early groups of sharks. And we may predict with almost the same degree of security that these forms will be found to picture the Cladoselachian in essential characters. For the Acanthodians, as we at present know them,²⁶ are obviously too specialized to have represented the ancestors of the line of Cladoselachians.

The causes of the extinction of the Acanthodians can, I believe, be suggested with a fair degree of probability. It is evident, of course, that the fin characters of this group premise great capability on the part of these fishes to dart forward, *i. e.*, in direct lines, a type of movement especially valuable when a definite kind of food is to be secured. On the

²⁵ Cf. *J. Morph.*, Vol. IX, pp. 110-111.

²⁶ Cf. the accompanying table.

CONTRASTED CHARACTERS OF CLADOSELACHIANS.	ACANTHODIANS.
<p>Smaller and more uniform in pattern throughout all regions. Practically unornamented. More gradual transition from shagreen denticles to sclerotic plates and to teeth. On anterior fin margins the denticles become large and concentrated, but do not produce spines. Denticles not specialized on the margins of lateral lines.</p>	<p>Large of size, varied in pattern, sometimes highly ornamented. In certain regions (head roof) individual scales become greatly enlarged and function as membrane bones. In general sharp contrast between shagreen denticles, teeth and sclerotic plates. High specialization of denticles margining sensory structures. Extraordinary development of spines, which may be regarded morphologically as collections of denticles enlarged and condensed on anterior fin margins (as suggested in the condition in the caudals of Acanthodians and in the paired fins of Cladose-lache), or as huge single denticles.</p>
<p>Arches similar, apparently, to Nofidanids.</p>	<p>Mandibular arch (Acanthodes) segmented like a branchial arch.</p>
<p>Notochordal. Neural and haemal elements heavy, not long and not connected with unpaired fins.</p>	<p>Notochordal. Neural and haemal elements shorter and more delicate than in Cladoselachian.</p>
<p>Both present; but small in size. The pelvic elements single, not conjoined (on the evidence of a new specimen of <i>C. kepleiri</i>).</p>	<p>Both present, but their structure obscured by the invasion of dermal elements. Pelvic element on each side as in Cladose-lache, but longer and more varied in outline. In Acanthodes "segments" (artifact?) present in pectoral arch, but in other genera these are absent.</p>
<p>Supporting basal and radial elements arranged similarly in paired and unpaired fins, concentrating anteriorward.</p>	<p>Spines supporting paired and unpaired fins. Caudal provided with radials as in Cladose-lache. Traces of radials in unpaired and paired fins.</p>
<p>Not well known. Eyes smaller (relatively) than in Acanthodians and less perfectly protected with dermal plates. Lateral line inconspicuous, open, apparently unbranched.</p>	<p>Eyes and auditory organs of large size. Marked branching of lateral line system. Usually but four (large) dermal plates protecting eye.</p>
<p><i>Sensory structures.</i></p>	
<p><i>Dermal defenses.</i></p>	
<p><i>Head.</i></p>	
<p><i>Vertebral column.</i></p>	
<p><i>Girdles.</i></p>	
<p><i>Fins.</i></p>	

other hand, it is very probable that they could not have altered quickly their direction, speed, or plane of movement, and they would thus be placed at a serious disadvantage when competing with ganoids, dipnoans, even with other sharks, for the capture of varied forms of prey. So, also, the teeth of Acanthodians indicate a smaller range for functional adaptation than the dentition of other sharks. Finally, the elaborate specializations of the integumentary defenses of Acanthodians lead in the direction of enlarging individual dermal cusps, instead of that of fusing the bases of the cusps. And the result was an unfavorable one. For the limit of the size of the individual cusp could soon be reached, while that of the fused basal plate (a true membrane bone) was greater and more plastic. Even the most perfectly armored Acanthodian, therefore, could not compete in this regard with the contemporary ganoids.

DESCRIPTION OF FIGURES.

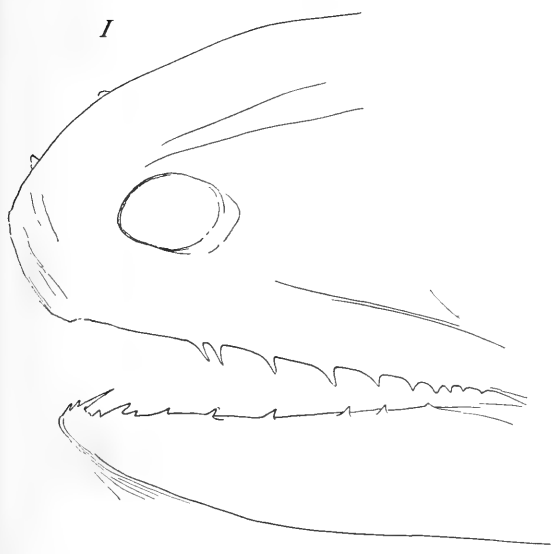
FIGS. 1-11. Teeth of Acanthodians. Figures of *Ischnacanthus*, excepting Fig. 11, which is of *Acanthodopsis wardi*.

FIG. 12. Jaw and branchial arches of *Acanthodes bronni*. 13. Jaw arches of *Ischnacanthus gracilis*. 14. Head-roof of *Climatius scutiger*. 15. Detail of a shagreen denticle from the head-roof of *Climatius reticulatus*. 16. Neural and hæmal arches of *Ischnacanthus gracilis*. 17. Neural arches and dorsal fin, *ibid.* 18. Pectoral spines and girdle of *Climatius reticulatus*. 19. Pectoral and pelvic girdles and spines of *Diplacanthus striatus*.

FIGS. 20-27. Fins and related structures in Acanthodians. 20. *Parexus falcatus*. 21. *Ibid.* 22. Caudal of *Diplacanthus tenuistriatus*. 23. Second dorsal of *Euthacanthus macnicoli*. 24. Dorsal of *Climatius uncinatus*. 25. Caudal of *Acanthodes bronni*. 26. Detached spine of *Climatius reticulatus*. 27. Shoulder girdle (half) of *Climatius falcatus*, showing dermal elements.

FIGS. 28-36. Sensory structures in Acanthodians. 28, 29. *Acanthodes bronni*. 30. *Climatius grandis*. 31, 32. *Ischnacanthus gracilis*. 33. *Climatius*. Detail of lateral line at a point anterior to second dorsal fin. 34. *Euthacanthus elegans*. Lateral line. 34a. Detail of a shagreen denticle margining lateral line. 35. *Acanthodes bronni*. Detail of lateral line. 36. *Euthacanthus macnicoli*. Detail of enlarged scales of ventral body-wall.

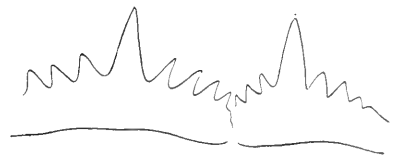
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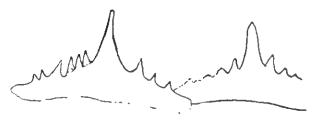
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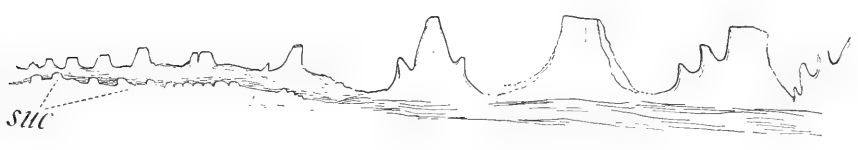
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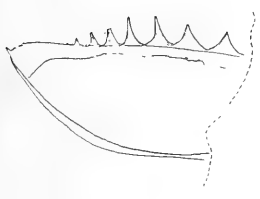
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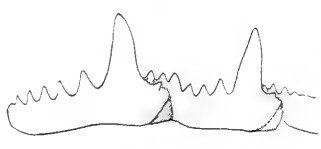
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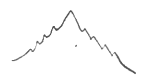
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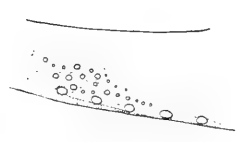
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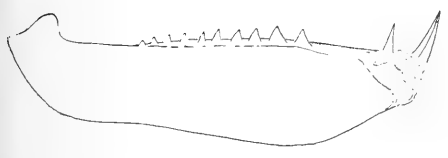
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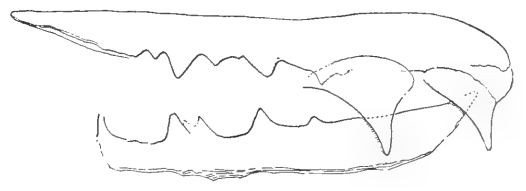
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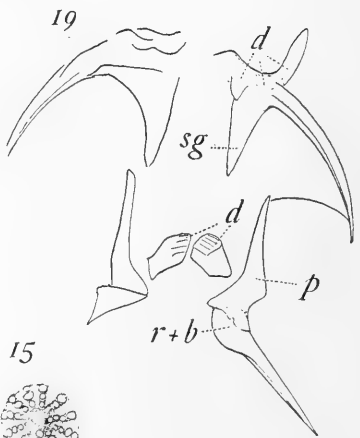
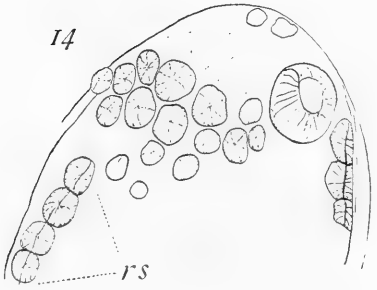
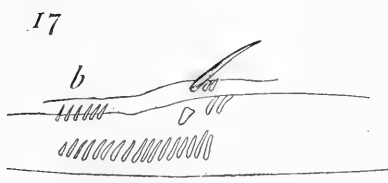
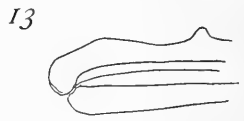
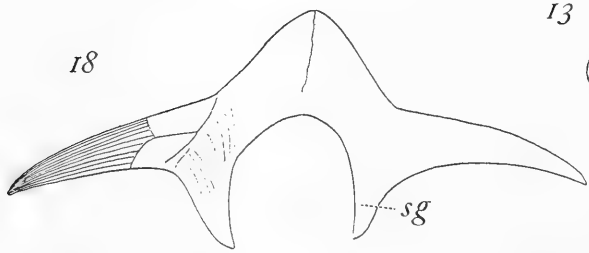
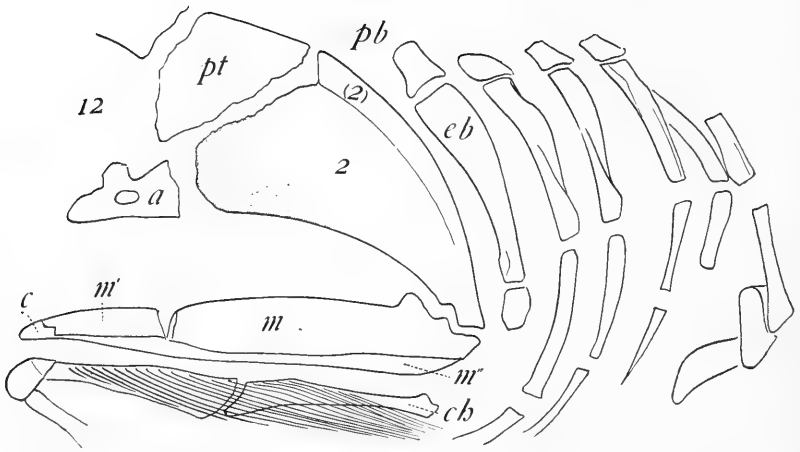


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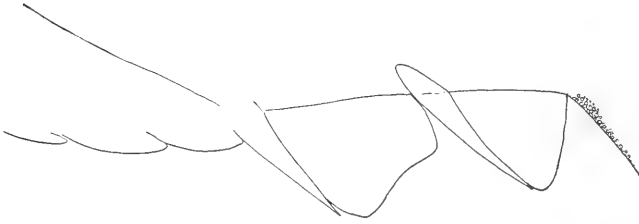
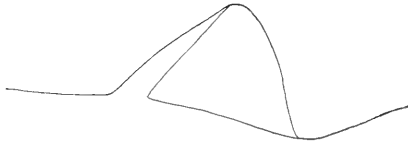


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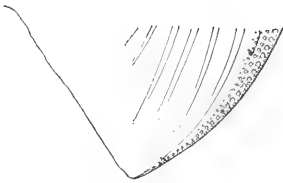
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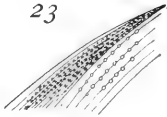
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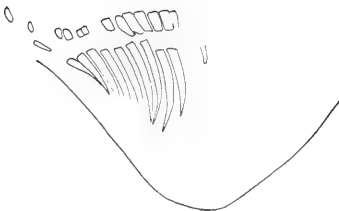
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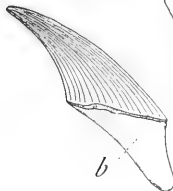
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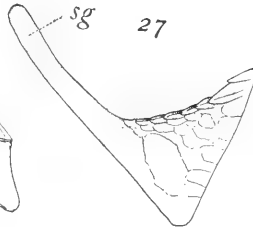
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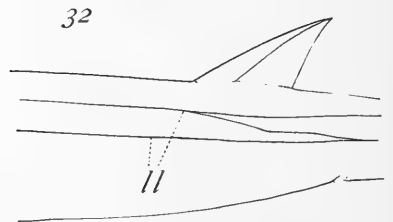
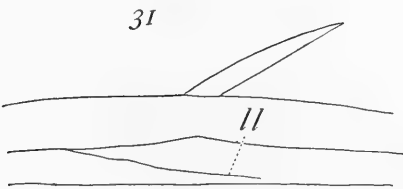
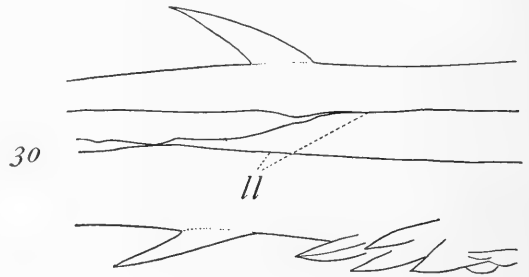
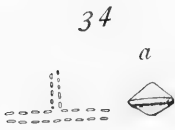
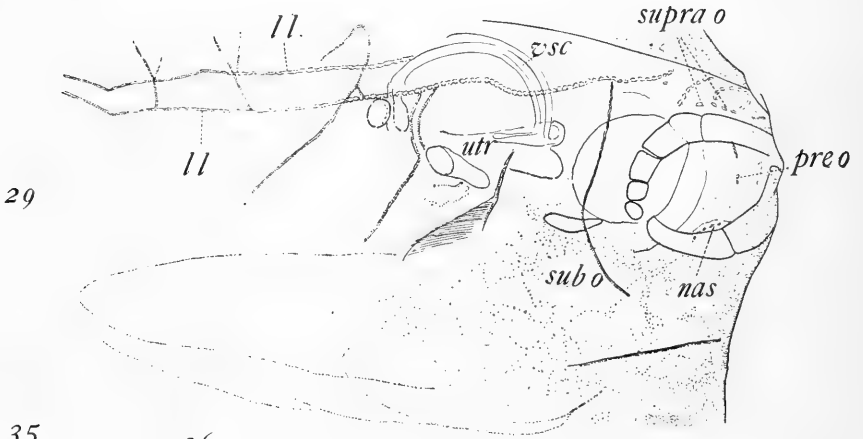
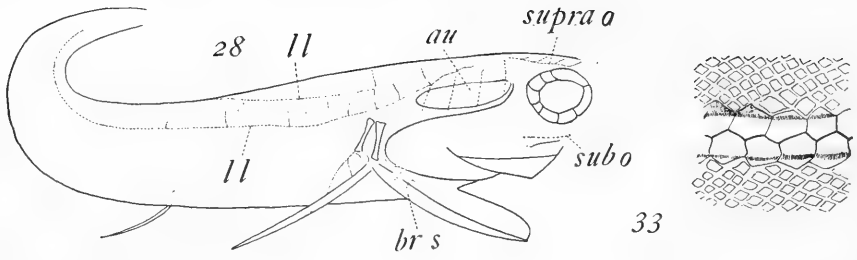


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RELATIONS OF THE FRONTAL LOBE IN THE MONKEY.

BY

E. LINDON MELLUS.

From the Anatomical Laboratory of the Johns Hopkins University.

WITH 20 FIGURES.

This communication covers the results of three experimental lesions upon the frontal lobe in the monkey. The animal employed in experiment A was the Bonnet monkey (*Macacus sinicus*), in the other two, B and C, the *Macacus rhœsus*. The object, in each case, was to sever the connection of the anterior segment of the internal capsule with the frontal lobe and, by the resulting degeneration, to trace, as far as possible, the route and destination of the fibers entering that segment of the internal capsule from the frontal lobe. A sub-dural aseptic incision was made in each case after the removal of a button of bone. After recovery from the anæsthetic the animal was carefully watched to observe any disturbance of function and killed after the expiration of 10 to 14 days, the brain cut in serial sections and subjected to microscopic examination.

EXPERIMENT A.

After removal of the button of bone from the left frontal region, the knife was passed through the dura directly into the substance of the brain in the direction of the median line. The external wound (Fig. 1, L. A.), was a perpendicular incision about 6 mm. in length, half-way between the pre-central sulcus and the frontal pole, crossed, at the junction of the middle with the inferior third, by the fronto-marginal sulcus, and at the same time dividing that sulcus into two equal portions. The animal recovered quickly from the ether and there was no evidence then or at any other time of disturbed function. Fourteen days after the operation the animal was killed with chloroform, the brain removed, hardened and stained by the Marchi method, and cut in horizontal sections.

Examination of the hardened brain showed the direction of the knife to have been inward and slightly downward, reaching little more than half-way to the middle line at the point of deepest penetration and

passing only through the cortex in its upper third. In the middle third of the lesion a small portion of the process of corona radiata extending into the anterior portion of the frontal lobe was severed from its connection with the central white mass, while in the lower third the lesion was again only cortical.

In the lower levels of the most anterior portion of the corpus callosum a few degenerated fibers crossed the middle line but could not be followed to their distribution in the right hemisphere. Most of the degeneration passed through the anterior segment of the internal capsule and ended in the anterior nucleus of the thalamus. Some degenerated fibers could be followed backward through the external capsule, around and through the posterior extremity of the lenticular nucleus. In this case these fibers could not be traced to their termination, but in the two following experiments their destination becomes quite clear, and the presence of these fibers in this case shows that at least a portion of them take their origin from the frontal lobe quite anterior to the lesions in the other cases. A small aggregation of degenerated fibers was present in the upper levels of the internal capsule, quite above the level of the lesion. These fibers could not be traced from the lesion, but that would be extremely difficult as the fibers are very fine and probably come in singly at different levels. They pass down with the capsular fibers, the number increasing from above downward as far as the lowest levels of the corpus callosum. In the lower levels of the capsule they are confined to the middle third of the posterior segment, from which they gradually disappear before reaching the crus. The striations of the lenticular nucleus showed traces of degeneration, more especially in the anterior portion of the nucleus. In the middle and lower levels of the lesion a few degenerated fibers were found running toward the occipital lobe in the optic radiation. There was no degeneration in the anterior commissure. No degeneration could be found in the right hemisphere.

EXPERIMENT B.

After the removal of the button of bone from the left frontal region the knife was passed directly into the substance of the brain, entering at a point just anterior to the genu of the pre-central sulcus (Fig. 1, L. B.). It was carried inward to a depth estimated to be sufficient to reach the middle line, and then downward and slightly backward to the base of the brain with the purpose of cutting off all fibres entering the anterior segment of the internal capsule as near as possible to the anterior extremity of the lenticular nucleus. The external wound was

7 mm. in length and its lower extremity reached and barely crossed the pre-central sulcus in the upper portion of the middle third of its ascending ramus. The hæmorrhage at the surface of the wound was very slight and was quickly and easily checked. The animal quickly recovered from the anæsthetic and though carefully watched there was no evidence of disturbed function. At the end of ten days it was killed with chloroform, the brain removed, hardened, and stained by the Marchi method and cut into horizontal sections.

Examination of the hardened brain showed that the operation had been followed by considerable hæmorrhage and consequent areas of softening. In the highest level the knife had passed quite through the corona radiata and reached the base of the cortex covering the mesial surface of the brain. 1 mm. below this level the knife reached the longitudinal fissure and, passing downward through the genu of the corpus callosum, amputated about 2 mm. of the left half from the anterior extremity of that structure (Fig. 3). Considerable hæmorrhage had taken place at this point, the blood working forward between the membranes and the cortex of the mesial surface of the left hemisphere almost to the frontal pole, and backward upon the upper surface of the corpus callosum within the longitudinal fissure. There were spots of softening in the cortex of the mesial surface of the left hemisphere just anterior to the lesion, not extending so far forward as the clot. There were spots of softening in the anterior and posterior extremities of the superior surface of the corpus callosum, but not extensive. The central portion of the corpus callosum was uninjured. There was slight infiltration and softening of the septum lucidum just anterior to the foramen of Monro. At one point in the level of the base of the external wound the blood had forced its way around the frontal pole and there were two small spots of softening in the cortex beneath the clot near the frontal pole. In the level of the foramen of Monro the point of the knife fell just short of the middle line, but entered the ventricle, into which there had been slight hæmorrhage, which was probably the cause of the softening in the septum lucidum mentioned above. In this level the wound passed through the caudate nucleus, severing the anterior fourth from the body of the nucleus (Fig. 4). Below the level of the foramen of Monro the line of incision drew nearer and nearer to the lenticular nucleus, and in the level of the posterior commissure just touched its anterior extremity, extending from the base of the cortex of the laryngeal area, just anterior to the process of corona radiata belonging to the Rolandic operculum, to the ventricle (Fig. 6). Just below these levels the incision fell short of the ventricle, and the hæmor-

rhage within the wound had been greater than in higher levels, but the resulting injury was confined to the tissues anterior to the line of incision. Throughout the whole extent of the anterior segment of the internal capsule the fibers entering it from the frontal lobe had been severed from their anterior connections. In the lowest levels the lesion extended only about half-way to the middle line, severing the process of corona radiata extending to the cortex of the lowest and most anterior portion of the Rolandic operculum and to the orbital surface of the inferior frontal convolution from its posterior connections.

One striking result of this experiment is the insignificant amount of degeneration in the severed end of the frontal lobe. There are a few fine degenerated fibers running forward from the lesion in most levels and there is an occasional fragment of a coarse fiber, but the mass of the corona radiata severed from the centrum semi-ovale shows no pathological change. In the upper portion of the centrum semi-ovale of the left hemisphere, somewhat above the level of the lesion, there is a mass of coarse degenerated fibers coming almost entirely from an area of softening on the upper surface of the corpus callosum, due to the pressure of a small clot. Most of the hæmorrhage came from an injured vessel in the great longitudinal fissure separating the hemispheres. This mass of degenerated callosal fibers was joined in various levels by degenerated fibers from the lesion, which could not, of course, thereafter be distinguished from the callosal fibers. After a short course downward a portion of these fibers was distributed to the cortex of the post-central convolution in the level of the lower extremity of the inter-parietal sulcus (sulcus retro-centralis inferior) (Fig. 2). The remainder of this degeneration in the centrum semi-ovale passed downward into the posterior segment of the internal capsule, where it was joined by coarse degenerated fibers from the lesion in the frontal lobe running backward through the anterior segment of the internal capsule. In the upper levels these coarse fibers were not grouped in their passage through the anterior segment, but below the level of the lower extremity of the fissure of Rolando (which is also the lower extremity of the external wound) and just before they disappeared from the section and gave place to finer fibers, they were confined to the portions of the anterior segment contiguous to the lenticular nucleus, and they mostly reached the posterior segment by breaking through the mesial projecting angle of the lenticular nucleus (Figs. 3, 4, and 5) quite outside those fibers composing the genu. These frontal lobe fibers, together with the remnant of degenerated callosal fibers, form a considerable group of scattered degeneration in the anterior half of the

posterior segment, but from this level downward they are passing into the thalamus faster than their places are taken by other degenerated fibers coming from the frontal lobe, so that below this level the amount of degeneration in the posterior segment of the internal capsule is constantly diminishing and has entirely disappeared when we reach the crus. These coarse fibers come apparently from the cortical area just in front of the external wound, corresponding to the center for the head and eye movements. Notwithstanding the extensive character of the lesion it is only in the lower levels of the external wound (studying the sections from above downward) that any considerable amount of degeneration can be traced from the wound backward. In the plane in which these sections were cut the lower extremity of the external wound corresponds to the lower extremity of the fissure of Rolando, the development of the anterior segment of the internal capsule and the appearance in the section of the foramen of Monro (Fig. 3). Degenerated fibers appear in the first bundles going to form the anterior segment and increase rapidly in number from above downward. The coarse and fine fibers gradually separate, the fine fibers being grouped upon the mesial side of the anterior segment, the coarse upon the external side next the lenticular nucleus. As stated above, this separation is barely accomplished when the coarse fibers disappear from the anterior segment. From this level downward copious fine degeneration passes backward from the lesion through the anterior segment until this structure disappears from the section and the posterior segment becomes the crus. In all these levels a small amount of fine degeneration passes backward from the lesion external to the lenticular nucleus in the external capsule to come to an end in the upper posterior portion of the superior temporal convolution. Although some of these fibers pass obliquely through the claustrum from the capsula extrema to the capsula externa, and *vice versa*, they do not apparently terminate in the claustrum. In lower levels (Figs. 6-9) the fibers passing backward, by way of the external capsule, turn around or break through the posterior angle of the lenticular nucleus, after which it becomes difficult to follow them with certainty, as they get mixed up with a lot of degenerated fibers coming from an area of softening in the splenium of the corpus callosum. A few fibers appear to run backward toward the occipital lobe in the optic radiation, but the majority seem to pass into and through the posterior segment of the internal capsule, and to end, in the sub-thalamic levels of the capsule, in the external geniculate body and the superior colliculus.

The coarse fibers, above alluded to, coming from the frontal lobe and

passing from the anterior segment *around* the genu to the posterior segment of the internal capsule, leave the capsule after a short downward course with the capsular fibers and pass into the external nucleus of the thalamus. Here they probably break up into collaterals or fine terminals, some of which end in terminal arborizations around the cells of the external nucleus, while others pass on as fine filaments which can be followed into the sub-thalamic region, where their course becomes confused with that of numerous fine fibers coming more directly from the anterior segment through the genu and anterior portion of the thalamus.

A few fibers from the frontal lobe, probably belonging to short association tracts, pass to the lower portions of both central convolutions. There was also some degeneration in the striations of the lenticular nucleus, especially in the upper portion. There was not a trace of degeneration in the caudate nucleus.

The great mass of degenerated fibers passing backward from the lesion is composed of fine fibers in the anterior segment of the internal capsule from the level of the foramen of Monro downward. In the upper levels a good deal of this degeneration appears to end in the anterior nucleus of the thalamus, but in lower levels it passes inward and backward through the thalamus, dividing at the fasciculus of Meynert into two tracts, the larger of which passes, mesial to that fasciculus, downward and backward into the gray matter surrounding the aqueduct of Sylvius (Figs. 6, 7, and 8). The smaller tract mingling with those fibers from the posterior segment of the internal capsule passes backward external to the fasciculus of Meynert and ends partly in the superior and partly in the inferior colliculus of the same side. Some of these fibers, also, find their way into the central gray matter surrounding the aqueduct. A few fibers pass around the aqueduct and cross the middle line as if to reach the superior colliculus of the opposite side (Figs. 9 and 10). No degenerated fibers appear to cross in the posterior commissure, although occasional fibers pass through it, at right angles to the transversely running fibers, to reach the superior colliculus. In the sub-thalamic region some of the degenerated fibers leaving the posterior segment of the internal capsule enter the capsule of the body of Luys, but it is impossible to say whether or not they end there.

EXPERIMENT C.

The lesion in this case was meant to be similar to that in the experiment just described, but the button of bone removed was a little posterior to the other and the external wound was nearly in the center of the pre-

central convolution (Fig. 1, L. C.). Its upper extremity was in the level of the genu of the pre-central sulcus, and it extended downward and backward 5 mm. about midway between the perpendicular ramus of that sulcus and the fissure of Rolando. There was no evidence of disturbed function and at the end of 10 days the animal was killed with chloroform, the brain removed, hardened, stained, and cut in horizontal sections like the others. Examination of the hardened brain showed that the lesion was much less extensive than in the preceding experiment, the resulting hæmorrhage much less, and the injury to the tissues in consequence of hæmorrhage much less. The wound nowhere reached more than two-thirds of the distance across the hemisphere toward the middle line, and, in the levels corresponding to the external wound, the lesion was almost entirely cortical. Owing to the oblique direction in which the pre-central sulcus sinks into the hemisphere in this level (Fig. 12), at a depth of 7 mm. (half the depth of the wound in this level) the knife reached this sulcus and separated a triangular portion of cortex from the pre-central convolution, and the most mesial extremity of the cut barely nicked the edge of the corona radiata. In lower levels, where the breadth of the hemisphere is greater and the depth of the cortex proportionately less, owing to the less acute angle of section, the lesion was less purely cortical and deeper tissues were involved. Below the level of the external wound the knife penetrated farther and farther into the corona radiata and at the point of deepest penetration the uppermost fibers entering the anterior segment of the internal capsule were completely severed (Fig. 13). This, however, is true of only the uppermost levels, for before the fissure of Rolando disappears from the section the incision falls short of the outermost edge of the anterior segment. Below this point the knife wounded the anterior portion of the putamen, and still lower severed the anterior portion of the claustrum from the rest of the nucleus, at the same time, of course, cutting all the longitudinal fibers in that portion of the capsula externa and extrema. Near the base of the brain the wound was again purely cortical, involving the anterior portion of the island and the operculum. At no point was that portion of the cortex injured which I have shown to contain giant cells.¹ A small clot had formed within the wound, damaging a small area of corona radiata, 2 x 4 mm. in extent, but there were no traces of hæmorrhage either from the wound on the external surface of the hemisphere or into the fossa of Sylvius.

In the upper levels of the lesion there was considerable degeneration

¹ A Study of the Location and Arrangement of the Giant Cells in the Cortex of the Monkey, *The American Journal of Anatomy*, Vol. IV, No. 4.

passing forward from the lesion into the frontal lobe, and backward into the centrum semi-ovale, to the cortex of both central convolutions in about equal amount, and backward among the fibers of the cingulum just beneath the cortex of the mesial surface (Fig. 11). Somewhat lower, but still in the level of the external wound, the degeneration in the pre-central convolution greatly exceeded that in the post-central. There was considerable degeneration in the anterior fourth of the corpus callosum, corresponding closely in antero-posterior extent to the tissue alluded to above as injured by the clot. It doubtless comes from that area, as the cut made by the knife could injure but very few callosal fibers. The majority of the degenerated fibers entering the upper levels of the anterior segment of the internal capsule are probably projection fibers having their cells of origin in that portion of the cortex of the pre-central convolution which lies within the pre-central sulcus just anterior to the external wound. A study of the serial sections shows that only a small number of these fibers—those having the longest course—can be motor fibers, and even that is open to question. Some of those fibers from the upper levels of the anterior segment enter the thalamus by passing mesial to the genu, others by passing through the genu; other fibers, mostly from slightly lower levels of the anterior segment, enter the anterior portion of the posterior segment in bundles that pass through the lenticular nucleus. These bundles leave the anterior segment at nearly a right angle, plunge into the lenticular nucleus and wind around the fibers of the genu to enter the posterior segment of the internal capsule. After a short downward course with the capsular fibers, some leave the capsule and enter the thalamus, where, together with those already described as entering the thalamus directly from the anterior segment, they break up into fine filaments, some of which probably end in either the external or anterior nucleus of the thalamus. A good many of these filaments, however, can be traced inward and backward to the ganglion habenulæ, where some of them appear to terminate about the cells of origin of the fasciculus retroflexus of Meynert.

In the level in which the ganglion habenulæ disappears from the section and gives place to the upper levels of the superior colliculus the passage of degenerated fibers from the lesion through the anterior segment of the internal capsule has practically ceased and the remainder of the degeneration in the posterior segment passes downward with the capsular fibers, but is gradually forced backward by the entrance of new fibers, toward the middle portion of the posterior segment. In this level also there is the beginning of a copious fine degeneration running back-

ward from the lesion in the capsula extrema and capsula externa, but mostly in the former, external to the claustrum. A little of this at first passes into the superior temporal convolution at a point almost directly underneath the lower extremity of the fissure of Rolando (compare Figs. 16 and 1). A much larger amount, perhaps most of the degeneration passing this way, reaches the occipital lobe by way of the optic radiation. The remainder of this degeneration, which is very considerable, passes around and through the posterior extremity of the lenticular nucleus, through the retro-lenticular segment of the internal capsule, and partly through the posterior portion of the posterior segment, into and through the thalamus, anterior to the pulvinar, to end in the superior colliculus. In lower levels a great deal of very fine degeneration running backward from the lesion, in the capsula extrema, passes inward, anterior to the geniculate bodies, to end in the inferior colliculus; some fibers running anterior to the crus pass into the middle third of it and mingle with the few degenerated fibers remaining of those which have come down through the internal capsule.

In the sub-thalamic region of the internal capsule the degeneration remaining in the posterior segment begins to pass out of the capsule into the sub-thalamic region. Many of these fibers pass into or through the capsule of the body of Luys; some pass through the centre median—some may possibly end in that nucleus—while the most mesial, corresponding to the most anterior in the posterior segment of the internal capsule, leaving the crus in successive levels, pass inward, backward, and downward into the central gray matter surrounding the aqueduct. These were the last of the degenerated fibers remaining in the crus.

IN CONCLUSION.

One important question presenting itself on a review of these degenerations is that of the origin of the mesial segment of the crus—the so-called fronto-pontine tract (frontale Brückenbahn, Flechsig). It has been very generally believed that the fibers of this tract come from the frontal lobe by way of the anterior segment of the internal capsule and end in the cells of the mesial pontine nuclei.

Ferrier and Turner report,² following ablation of the frontal lobe in the monkey, degeneration in the anterior segment of the internal capsule in the most mesial and ventral parts, immediately above the anterior com-

² Phil. Trans. Royal Soc. of London, 1898.

missure, in the mesial tract in the crus and in the corresponding portions of the pons, which disappeared before reaching the lower levels.

Von Monakow's case³ of an extensive destruction of cortex of contiguous portions of the second and third frontal convolutions shows almost complete destruction of the mesial segment of the crus, but it is not clear that there is much degeneration in the anterior segment of the internal capsule. In this case there is also extensive destruction of sub-cortical tissue, which complicates the question of the origin of the fibers degenerated in the crus.

Dejerine⁴ believes that the fibers of this tract arise in the cortex of the frontal and Rolandic operculum, but suggests that some of its fibers may come from the orbital surface of the third frontal convolution. He bases his conclusions upon the study of degenerations following such extensive lesions that the possibility of error does not seem to be excluded.

These experiments seem to show that the mesial segment of the crus does not contain any cortico-pontine fibers coming by way of the anterior segment of the internal capsule. In an extensive series of experimental ablations of small areas of cortex in the Rolandic and frontal operculum in the monkey (Fig. 20), I have never observed any degeneration in the mesial segment of the crus. Degenerations from such lesions always enter the internal capsule in levels above the highest levels of the anterior segment. In all my experimental ablations of the so-called facial area of the cortex the degenerated fibers have been found in the posterior segment of the internal capsule.⁵ The genu of the internal capsule, like the mesial segment of the crus, was entirely free from degeneration. It can hardly be claimed, as yet, that it has been shown that the fibers of the mesial segment of the crus constitute a definite tract arising in limited areas of the cortex of the frontal lobe. If, as is claimed by Dejerine, the crus is made up of cortical projection fibers only, the cells of origin of the mesial segment will be found in the cortex either of the frontal lobe or of the island of Reil. I think Dejerine wrong in the statement that the fibers of the genu ("*véritable faisceau géniculé*") come from the anterior segment (Vol. 2, p. 30). In his "*Cas Morceau*" (Vol. 2, p. 148), the anterior segment of the capsule is apparently completely destroyed,

³ Nothnagel: *Gehirnpathologie*, Vol. IX, Wien, 1897.

⁴ *Anatomie des Centres Nerveux*, Tome II, Paris, 1901.

⁵ *Experimental Degeneration Following Unilateral Lesions of the Cortex Cerebri*, Proc. Royal Society of London, Vol. LVIII. *Motor Paths in the Brain and Cord of the Monkey*, Journal of Nervous and Mental Diseases, April, 1899.

but there is no degeneration in the genu of the same side, and, of course, the corresponding mesial segment of the crus is uninjured. On the contrary, in "Cas Schweigoffer" (Vol. 2, p. 135), by which he appears to prove that the origin of the fibers of the genu and of the mesial segment of the crus is in the cortex of the frontal and Rolandic operculum, the conditions are quite reversed. The genu of the internal capsule and the mesial segment of the crus are completely destroyed, while there is but slight evidence of degeneration in the anterior segment of the internal capsule. In this case it seems quite evident that but little of the degeneration in the genu could have come from the anterior segment. Here, as well as in the case reported by v. Monakow, it seems quite reasonable to suppose the degeneration in the genu and the mesial segment of the crus may have been dependent upon the destruction of sub-cortical tissue in the island of Reil and that these fibers may have their origin in the cortex of the upper and anterior portion of the island. V. Bechterew,⁶ on the authority of Zacher, suggests such an origin, and many findings point in that direction. Fibers from that cortical area would find their way to the genu over and through the thin crest of the lenticular nucleus.

Another point worthy of special notice in these experiments is the passage of fibers from the frontal lobe directly through the thalamus to the gray matter surrounding the aqueduct. Some of these pass from the anterior segment of the internal capsule directly into the thalamus; others, sweeping around the genu, through the globus pallidus, reach the posterior segment of the internal capsule, and, after a shorter or longer course with the capsular fibers, pass into and through the thalamus to the same destination. Some of these fibers (perhaps all) seem to come from the pre-Rolandic cortex of the frontal lobe, and their origin and course suggest that they may rise in cortical areas representing eye movements, and that they take this course as the shortest route to the nuclei of the oculo-motor nerves. It also has the advantage of furnishing collateral connection with the nuclei of the thalamus, sometimes supposed to be active agents in emotional reflexes (Bechterew). It will be noticed that the coarser of these fibers follow the capsule into the crus. These fibres also find their way to the gray matter around the aqueduct after leaving the crus.

One set of fibers degenerating in these experiments suggests some rather unexpected connections of the frontal lobe with the basal ganglia—that is,

⁶ *Leitungsbahnen im Gehirn und Rückenmark*, 2d German Edition, Leipzig, 1899.

the fibers passing mostly outside the claustrum and turning around the posterior extremity of the lenticular nucleus. That many of these fibers running backward beneath the cortex of the island pass to the temporal and occipital lobes is well known and this explains many clinical manifestations. But we appear to have here at least four tracts:

1. That going to the superior temporal convolution;
2. That going to the occipital lobe;
3. That just referred to as turning inward around the posterior angle of the lenticular nucleus and joining the fibers from the posterior segment of the internal capsule on their way to the central gray matter around the aqueduct; and
4. A group of fibers passing inward and downward from the area of Wernicke to end in the superior colliculus.

ABBREVIATIONS USED IN THE FIGURES.

- A.* = Aqueduct of Sylvius.
Aff. S. = Affenspalte.
A. P. F. = Anterior pillar of the fornix.
C. A. = Anterior commissure.
C. C. = Corpus callosum.
C. L. = Body of Luys.
C. P. = Posterior commissure.
E. G. = External geniculate body.
F. M. = Foramen of Monro.
F. R. = Fasciculus retroflexus of Meynert.
G. H. = Ganglion habenulæ.
Gl. P. = Pineal gland.
I. G. = Internal geniculate body.
I. P. = Interparietal sulcus.
L. = Lesion.
N. A. = Anterior nucleus of the thalamus.
N. C. = Nucleus caudatus.
N. M. = Centre median.
N. R. = Red nucleus.
N. T. = Trochlear nucleus.
P. = Pulvinar.
P. C. I. = Pre-central sulcus, descending ramus.
R. = Fissure of Rolando.
R. III. = Root-bundles of third nerve.
S. = Fissure of Sylvius.
S. T. = Temporal sulcus.
V. = Ventricle.
V. d' A. = Column of Vicq d' Azyr.

DESCRIPTION OF FIGURES.

FIG. 1. Outline drawing of external surface of left hemisphere, showing the line of incision in the three experiments. *L.* = Lesion in A, B, and C. The horizontal lines represent the plane of section in the figure corresponding to the numeral at the end of each line.

FIGS. 2 to 10 inclusive show the degenerations in Experiment "B."

FIGS. 11 to 19 inclusive show the degenerations in Experiment "C."

The small scale (natural size) upon which these outline drawings are produced requires much exaggeration of the lines representing degenerations. They therefore only represent the location and direction of the degenerated fibers.

FIG. 20. Outline drawing of external surface of the left hemisphere, showing areas in the cortex of the frontal and Rolandic operculum which have been extirpated by the writer without producing any degeneration in the genu of the internal capsule or in the mesial segment of the crus.

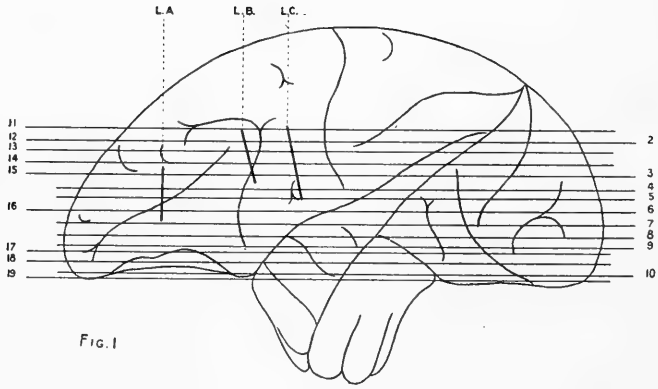


Fig. 1

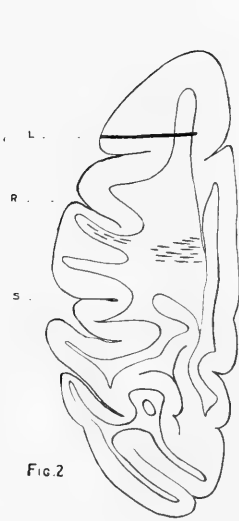


Fig. 2

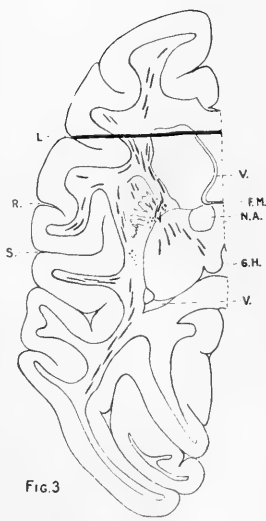


Fig. 3

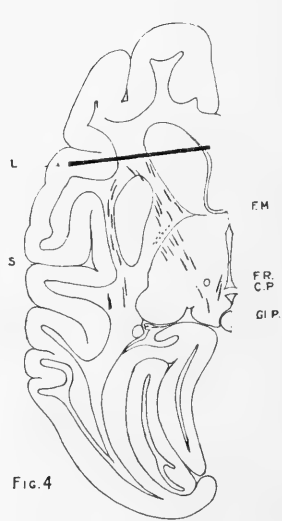
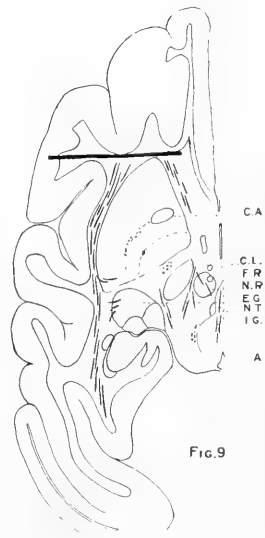
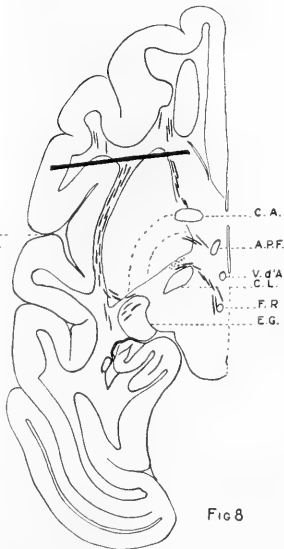
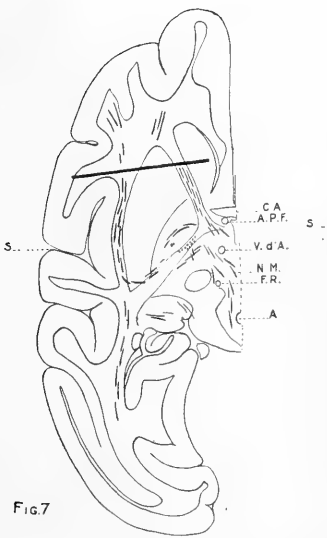
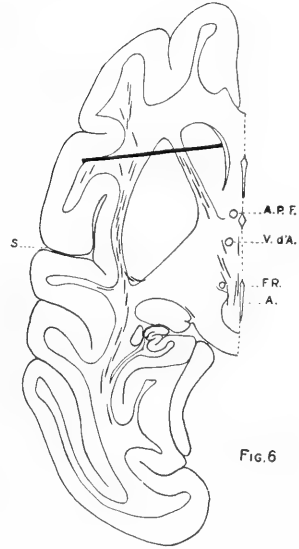
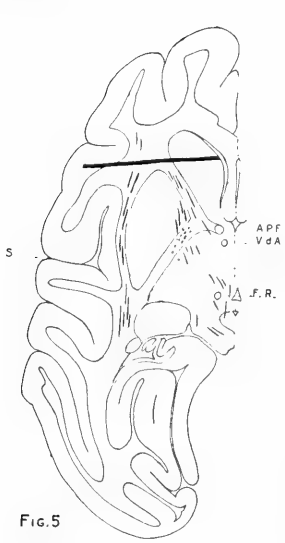
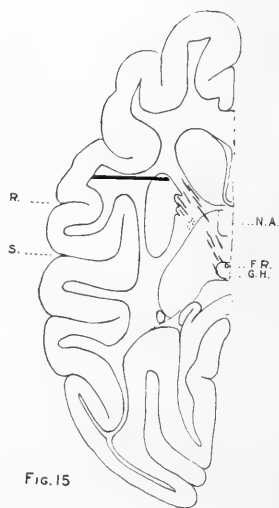
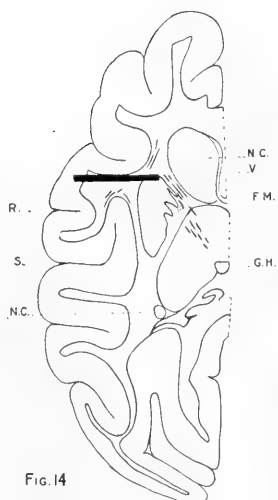
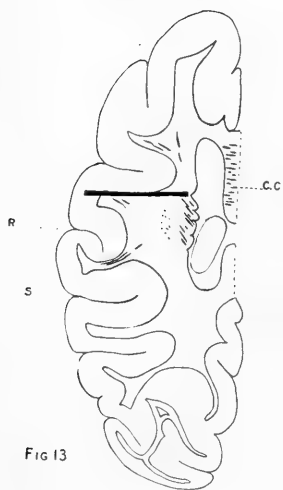
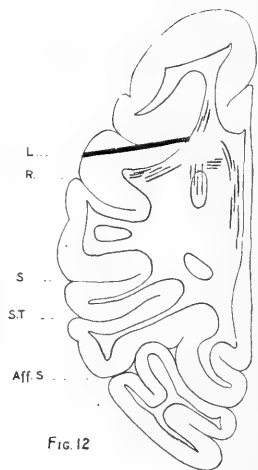
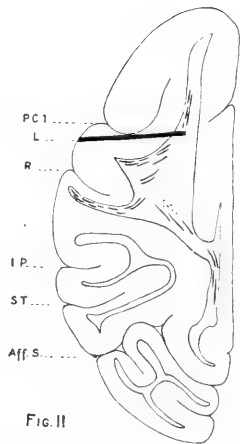
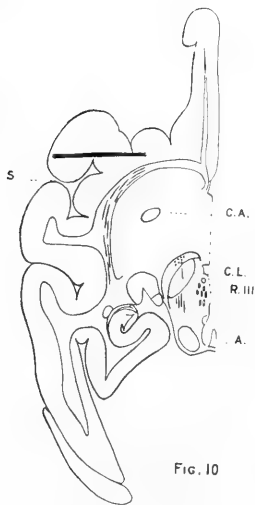


Fig. 4





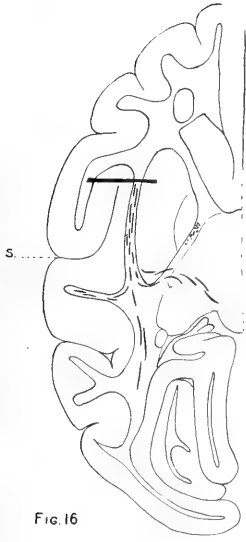


FIG. 16

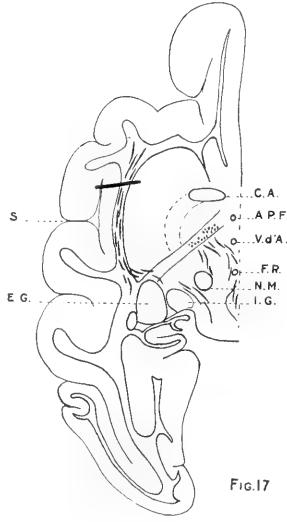


FIG. 17

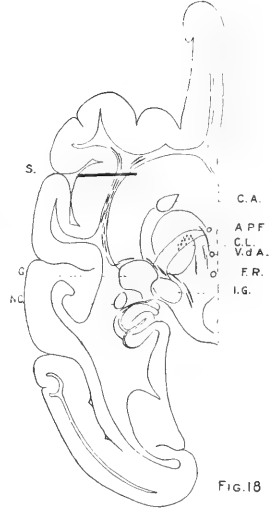


FIG. 18

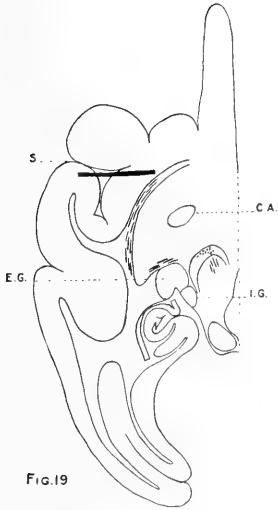


FIG. 19

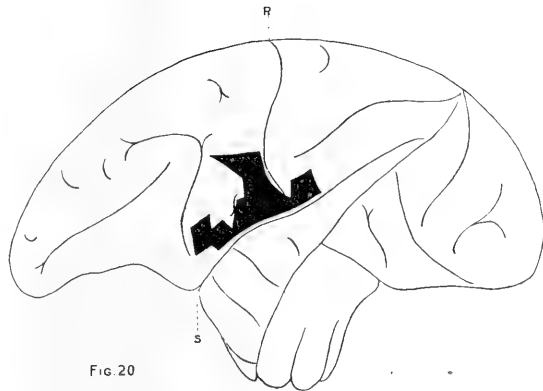


FIG. 20

A RESTUDY OF THE MINUTE ANATOMY OF STRUCTURES
IN THE COCHLEA WITH CONCLUSIONS BEARING
ON THE SOLUTION OF THE PROBLEM OF TONE
PERCEPTION.

BY

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WITH 2 PLATES.

It is generally conceded that in the cochlea is located the mechanism whereby the physical phenomena of sound waves are converted into the nerve impulses which result in tone perception. Long ago the idea was suggested that this process for the several tones takes place in different parts of the cochlea. It remained for Helmholtz, however, to give this hypothesis its greatest scientific support, and in literature it is best known as the "Helmholtz Theory" or the "resonator theory" of tone perception.

This theory finds a powerful support in the physiological fact that the organ of hearing possesses the faculty of tone analysis, a phenomenon that has its physical analogy in the sympathetic vibrations which take place in the strings of a piano forte when the corresponding tones are produced in another instrument. The clinical observation, too, that in certain cases where there has been a more or less extensive destruction of the function of hearing, circumscribed "islands of hearing" are found where the perception for certain tones is preserved, speaks strongly in favor of the presumption that the perception for the various tones takes place in separate and distinct parts of the cochlea.

Anatomically, we find in the long rows of hair cells running throughout the entire length of the cochlea a mechanism admirably suited to the requirements of such a theory. These hair cells are the real end-organs, wherein the transference of the physical sound waves to nerve impulses takes place. Each cell, or rather group of cells, when adequately stimulated, leads to the perception of a particular tone. The higher tones, presumably, are taken up by the cells located in the basal coil, the lower tones by the cells near the apex of the cochlea.

The manner in which the stimulus is applied to these hair cells and

especially how certain cells are stimulated by impulses coming from particular tones only and not from others; these are problems in the resonator theory which occupied the attention of Helmholtz and have been the subject of investigation by the physiologists who have since made a study of the question of tone perception.

The structure of the hair cells themselves suggests the probable way in which a stimulus is applied to these cells. Each cell is provided with a clump of short hairs which project from its free end. The stimulation of these cells is obtained presumably when these hairs are brushed against the overhanging membrana tectoria.

The difficult problem in the resonator theory is to determine how certain hair cells are stimulated by certain tones only while other cells are stimulated by other tones. Helmholtz was at first inclined to attribute to the rods of Corti this selective function of resonators. When it was shown that birds and crocodiles lack these rods, Helmholtz gave up this idea and fixed upon the radiating fibers of the membrana basilaris as the real resonators of the organ of Corti. These fibers, estimated from 15,000 to 25,000 in number and varying in length from 0.04 mm. in the basal coil to 0.5 mm. at the apex of the cochlea, should vibrate, according to Helmholtz, each for a particular tone. In vibrating, the superimposed hair cells presumably would be carried upwards and their projecting hairs brushed against the membrana tectoria.

Objections to this view of the function of the membrana basilaris have been expressed. In the first place, the tone differences that can be recognized by different individuals are by no means the constant factors we would expect to find in case a distinct anatomical entity, a separate fiber of the basilar membrane, was endowed with the ability to vibrate each in response to a distinct tone. A trained musical ear is capable of recognizing a much smaller difference between tones than an untrained ear. Some, at least, of the difficulties in his theory were appreciated by Helmholtz himself as, for example, the question whether it is possible for fibers as short as those of the basilar membrane to be thrown into vibration at all by sound waves. Ewald recently, in an ingeniously constructed ear model, has been able to demonstrate that a stretched rubber membrane, measuring 0.5 mm. broad, could be made to vibrate, when suspended in water, by sound waves conducted from the air. Such a demonstration, however, falls far short of proving that the much shorter, thicker, and more rigid membrana basilaris could respond in a similar manner. It must not be lost sight of in this connection that the cells forming the organ of Corti are not placed separately on the

basilar membrane but are placed in contiguity, one with the other, thus forming a continuous whole, and that the thickness of the vibrating membrane is more accurately represented by a structure which includes the thickness of the fibers of the membrana basilaris plus the thickness of the superimposed cells as well (see Fig. 1). Again, the several radiating fibers of the membrana basilaris are not strung independently but they all form part of a continuous membrane and if thrown into vibration would not vibrate independently of the adjacent fibers. This fact, indeed, was recognized by Helmholtz and utilized in explaining the phenomena of "beats."

Additional evidence that this is not a vibrating membrane is found in the fact that the base of the inner rod of Corti rests on the firm structure of the labium tympanicum. This fact was recognized by Kuiles, who still retained the idea, however, that this is a vibrating membrane and explained that the vibrations take place about the base of the inner rod as a fulcrum, thus brushing the hair cells to and fro against the membrana tectoria.

In making a study of the structure of the membrana basilaris in the various parts of the cochlea, I have come across conditions which I believe demonstrate that this membrane cannot be the vibrating structure which it was believed to be by Helmholtz. In tracing the basilar membrane through carefully made sections near the beginning of the lower coil in the vestibule, I found that this membrane, at a considerable distance from the point where the cochlear tube begins, became so thick and rigid as to preclude any idea of its being a vibrating structure. This is well shown in Fig. 1, which is taken from the labyrinth of a new-born pig. In a labyrinth taken from another pig, I found in this part of the cochlea complete absence of any structure that could properly be called a basilar membrane (see Fig. 2). Here the crista of the ligamentum spirale, as seen in a section, tapering gradually to a point, is attached directly to the labium tympanicum. There is no basilar membrane and the perfectly formed organ of Corti rests on the stiff, rigid structure of the crista of the spiral ligament. In another preparation, a solid bony plate connected the lamina spiralis ossea with the outer wall of the cochlea at a point in the lower end of the basal coil where a perfectly formed organ of Corti was still present (see Fig. 3). The fact that the disappearance of the membrana basilaris as a possible vibrating structure takes place at a point in the lower end of the basal coil where the organ of Corti is still perfectly formed points to the conclusion that the vibration of this membrane is not essential for hearing. On the other hand, the fact that the

disappearance of the cells forming the organ of Corti and of the membrana tectoria takes place simultaneously is strong, presumptive evidence that the latter structures alone constitute the mechanism essential for the function of hearing.

Since these preparations show conclusively that the stimulation of the hair cells of the organ of Corti in this part of the labyrinth cannot be accomplished through a vibrating membrana basilaris, it is not logical to assume that in other parts of the cochlea, where the membrana basilaris may appear capable of vibrating, the stimulation of the hair cells must be accomplished through this means.

In giving up this conception of the Helmholtz theory that the basilar membrane is a vibrating mechanism, the question arises: May not the several hair cells or groups of these cells when stimulated give the perception of particular tones, each acting as its own agent in selecting as its stimuli only such impulses as are produced by a particular tone? An objection to this hypothesis is the fact that anatomical study fails to discover those differences between the several cells which we might expect to find in case they possessed this varying function. A more serious objection, however, is the fact that this hypothesis fails to account for the peculiar secondary phenomena of tone perception which are explained so ingeniously by the resonator theory. The fundamental principles in the resonator theory of Helmholtz still remain as the most plausible explanation of the various phenomena of tone perception. With the physiological and clinical facts which we possess, we can hardly escape accepting a resonator theory in some form.

Moreover, if we assume that the several hair cells act in this way and yet accept the idea so plainly indicated by the structure of these cells, namely, that the transference of impulses to them is accomplished through the stimulation of their projecting hairs, then the question arises, can these hairs be stimulated directly by impulses in the endolymph without the intervention of the membrana tectoria? No fundamental objection to this theory is apparent, provided we can accept the anatomical relations usually given as existing between the membrana tectoria and the hair cells, namely, that this membrane floats in the endolymph free above these cells. The fact that this hypothesis fails to recognize any function for the membrana tectoria is, however, an objection to it.

In an investigation into the character and relations of this most interesting membrane, I have been convinced that the tectorial membrane does not float free in the endolymph above the cells forming the organ of

Corti. The membrana tectoria has always proved to be the most elusive of all the delicate structures of the membranous labyrinth to study. Its delicate semi-fluid substance is such that it is most readily distorted by the fixing and hardening agents necessary in preparing the labyrinth for histological study. Our ideas of the character and relations of this membrane must be made up from a study of these distortions since a membrana tectoria in its normal relations that has not been acted upon by fixing and hardening agents has never been studied. A conception of the variety of distortions which this delicate film-like structure undergoes in the process of preparation for histological study can be got by glancing over the different drawings of this membrane that have appeared in the published works on the labyrinth. In studying the membrana tectoria in preparations from a large number of labyrinths, I am certain that this membrane does not float free in the endolymph above the organ of Corti, but that this position, which is the one usually described, is the result of the shrinking of this structure owing to the fixing agents. This fact is quite evident from the study of such preparations as the one shown in Fig. 4. Here, although more or less shrunken and distorted, the membrana tectoria is still found attached to the organ of Corti. The explanation of the spiral band found on the under surface of the membrane, which is known as the "Streifen of Hensen," is here made clear. It is a sort of facet where normally the membrane is attached to the supporting cells just internal to the inner row of hair cells. In this particular preparation, the membrane is only partially lifted from its normal position and the "Streifen of Hensen" is still glued to the supporting cells of the organ of Corti, which have in turn been somewhat pulled out owing to the partial withdrawal of the membrana tectoria. Another fact shown in this preparation is that the hairs of the hair cells project into the under surface of the tectorial membrane. That these hairs actually penetrate the membrana tectoria and are not merely brought into touch with it by accident is well shown by the preparation in Fig. 5. Here the membrana tectoria has been partially torn away from its moorings, so much so, indeed, that the "Streifen of Hensen" is no longer in contact with the supporting cells and the membrane has also been detached from the inner hair cell. The hairs of the three outer hair cells, however, are still inserted into the tectorial membrane, which, as the result of shrinking, has suffered a distortion in its delicate structure which could result only from a more or less close adherence of the hairs to this membrane. This relation between tectorial membrane and

hair cells prevents the impulses in the endolymph from coming in direct contact with the hair cells and, therefore, invalidates the hypothesis that these cells may act as their own agent in selecting their stimuli directly from the impulses passing through the endolymph. The conclusion to which these facts logically lead is that the stimulation of the hair cells is accomplished only by the vibration in the membrana tectoria transmitted to it by the impulses passing through the endolymph. The membrana tectoria must, therefore, be the mechanism which mediates impulses passing through the endolymph to the hair cells.

A consideration of the following facts renders this theory much more plausible than the hypothesis that this function is accomplished by the vibration of the membrana basilaris. The sound waves transmitted through the membrana tympani and the chain of ossicles produce impulses first in the labyrinthine fluid contained in the vestibule. It seems much more probable that these impulses, passing into the scala vestibuli, impinge directly upon the membrana tectoria, producing vibrations in it, since the delicate membrana vestibularis in no way hinders the passage of these impulses to the endolymph, than to assume that they must pass through the entire length of the scala vestibuli, then through the helicotrema into the scala tympani and then back again through the entire length of the cochlea in order to carry the basilar membrane upwards and push the hair cells against the membrana tectoria. Physically, the delicate semi-fluid structure of the membrana tectoria renders it admirably suited for responding to the most delicate impulses passing through the fluid in which it is suspended. Quite the opposite is true of the membrana basilaris, the fibers of which are so short and rigid that even in its most favorable part this has always been recognized as an objection to the idea that it could be a vibrating structure,—an objection first raised by Helmholtz himself.

A striking characteristic of the membrana tectoria when examined throughout the several coils of the cochlea is its great variation in size from one end of the cochlea to the other. This is well illustrated in Figs. 6 to 10 which have been drawn accurately with the aid of the camera lucida from sections of different parts of the cochlear tube of a single labyrinth. It is seen at a glance that the size of the membrana tectoria near the apex of the cochlea is many hundred times its size near the beginning of the basal coil. Beginning with the lower end of the basal coil there is a gradual increase in the size of the membrana tectoria until the apex of the cochlea is reached. Another characteristic which this

membrane possesses is its lamellar structure. An immense number of delicate lamellæ are found taking their origin from the portion of the membrane which rests on the labium vestibulare. The lamellæ, more compact where they converge along the dorsum of the membrane, curve gracefully outward and downward towards the lower border of the membrane. These lamellæ give the membrana tectoria somewhat the appearance of a soft feather. They vary in length with the varying size of the tectorial membrane, the shorter lamellæ occurring near the beginning of the basal coil, and the longest at the apex of the cochlea. They are supported and held together by an apparently homogeneous semi-fluid substance. The specific gravity of this substance appears to be the same as that of the endolymph in which the membrane is suspended. This is shown by the fact that, in spite of its semi-fluid character, it retains its position in the endolymph though supported only by its contact with the labium vestibulare and where the "Streifen of Hensen" is in contact with the supporting cells of the organ of Corti. The use of hardening and fixing agents always produces decided distortions of the membrana tectoria as the result of shrinking. This shrinking is not evenly distributed throughout the membrane but varies evidently owing to the unequal distribution of the lamellæ. The most marked shrinking taking place where the lamellæ are the most compact, that is, along the dorsum of the membrane and toward the end which is attached to the labium vestibulare. This results in a certain more or less characteristic distortion of the membrana tectoria which is often observed in our best preparations otherwise free from distortions. This characteristic distortion is the pulling of the membrana tectoria back towards its attachment at the labium vestibulare, and away from the hair cells. The result is that often in otherwise perfect preparations the membrana tectoria does not lie in contact with the hair cells but is lifted up and pulled back toward the modiolus.

These characteristics of the membrana tectoria render it suitable to respond to the most delicate impulses passing through the endolymph. The great variation in size of the membrane from one end of the cochlea to the other, together with its lamellar structure, are physical characteristics which suggest the probable basis for a series of resonators which make it possible for the membrane in one part of the cochlea to respond to impulses of a certain pitch and in another part to impulses of another pitch. In this way the small impulses produced by the high pitched tones would in some way set to vibrating the tiny tectorial membrane found in the beginning of the basal coil, while the larger impulses arising

from the deeper tones would produce vibrations in the much larger tectorial membrane found in the upper coils.¹

The vibrations in a part of the *membrana tectoria* produced by a particular tone must necessarily involve a considerable area of this structure. As a result, a more or less extensive group of hair cells is stimulated. The nerve impulses arising from the stimulation of the several hair cells included in this group come together in the brain center of the cortex, where the tone picture forms the final step in the perception of this particular tone. When a tone slightly higher or lower than this one, is produced, the same group of hair cells is stimulated, excepting for the addition of a few more cells at one end and the loss of a few cells at the other end of the area involved. The sum total of the impulses which reach the center in the brain is, therefore, different for every tone, however near they may be in the scale. The ability to distinguish between the several tones is, therefore, a matter of education, since anatomically we encounter no difficulty in accounting for a different tone picture for each particular tone difference.

A necessary corollary of the fact that the vibration of the *membrana tectoria* resulting from a particular tone spreads over a considerable area of this membrane is the fact that when two tones near each other in the scale are produced simultaneously the two areas of the *membrana tectoria* thrown into vibration will overlap to a greater or less extent. This overlapping of the areas stimulated by several tones was first conceived by Helmholtz in his basilar membrane theory and has been looked upon by many as one of the strongest arguments in favor of his resonator hypothesis since it offers perhaps the most plausible explanation of the most difficult of all the secondary phenomena of tone perception to be accounted for, namely, the phenomenon of "beats." The objections to this ingenious conception of Helmholtz which are based on the very apparently rigid inflexible character of the *membrana basilaris* do not

¹ Whether the facts which we now possess regarding the structure of the *membrana tectoria* are accurate enough to furnish the physical basis which will explain exactly how the *membrana tectoria* fills the role of resonator is a question for the physicist to answer. Whether or not the anatomist will ever be able to furnish accurate enough data regarding the structure of the *membrana tectoria* to satisfy in detail the physical requirements of a resonator, at least of the types of resonators now recognized by the physicists, does not in any way detract from the logical conclusion here reached, viz. that in the *membrana tectoria* is located the mechanism which fills the role of physical resonator.

hold when the delicate film-like, semi-fluid *membrana tectoria* takes the rôle of resonator.

Again, the peculiar alterations in the function of hearing which occur as the result of pathological changes in the cochlea, find a more ready explanation on the hypothesis that the *membrana tectoria* is the resonator than that the *membrana basilaris* fills this rôle.

In the first place, the occurrence of "tone islands," that is, the preservation of certain circumscribed areas of hearing in cases where there has been more or less extensive destruction of hearing, finds as ready an explanation with the *membrana tectoria* as resonator as when we attribute this function to the *membrana basilaris*.

In the second place, the explanation of the pathological phenomenon known as "*diplocousis binauralis dysharmonica*," where the patient hears a tone in the affected ear of a pitch different from that heard in the normal ear, is much more readily accounted for on my hypothesis. This peculiar phenomenon is readily explained as due to some slight alteration in the delicate and easily disturbed *membrana tectoria* which causes it to respond over a certain area to impulses of a different pitch than when in the normal condition.

Finally, an explanation of "*tinnitus aurium*" or subjective noises may possibly be found in this conception of the structure and function of the *membrana tectoria*. In the first place, the character of *tinnitus aurium* is usually that of an indefinite sound like the wind in the forest or the rushing of water, sounds made up of a great complexity of tones and with no definite pitch. Clinically, these subjective sounds arise from a variety of pathological conditions. One of the best known causes of *tinnitus* is pressure applied to the conducting apparatus so as to push the foot plate of the stapes into the oval window. This results in *tinnitus aurium* of the indefinite character described above. What actually takes place when the stapes is thus forced into the oval window is an increase in the pressure of the intralabyrinthine fluid. The result of this alteration in pressure must be a disturbance of the *membrana tectoria* which has apparently the same specific gravity as the endolymph when the latter is under its normal pressure. The hairs from the hair cells, as I have shown above, normally penetrate into the lower surface of the *membrana tectoria*. Any disturbance in this membrane, however slight, would, therefore, alter the normal relations existing between the membrane and the hair cells. It seems that such an alteration from the normal relation between *membrana tectoria* and the hairs of the hair cells would constitute a stimulation of these cells. When the foot plate

of the stapes is pushed into the oval window there would result a slight stimulation of perhaps all the hair cells in the cochlea. The result would be exactly what we meet with clinically a tinnitus aurium of an indefinite character like the wind in the forest or the roar of a sea-shell. When a sudden increase or decrease in the blood-pressure results in tinnitus aurium the cause is the same as when the stapes is pushed into the oval window. The explanation for the increase or decrease of the intralabyrinthine pressure is here quite evident. The tinnitus aurium arising from the administration of certain drugs is also plausibly explained in the same way as due to an alteration in the blood-supply to the labyrinth with resulting alteration in the pressure of the intralabyrinthine fluid. The tinnitus occurring in Menier's disease, where there has been an escape of blood into the cochlea, is also similarly accounted for by this conception of the physiology of tone perception. The disturbances in the function of hearing arising from an injury produced by a shrill whistle or an explosion near the ear are also readily explained. In the first place, when a permanent disturbance in hearing is thus produced it can be readily accounted for by a partial severance of the relation between membrana tectoria and hair cells so that the hairs from a greater or smaller number of these cells project free in the endolymph and do not come in contact with the membrana tectoria and, therefore, cannot receive the stimulation from impulses passing through the endolymph. On the other hand, when there results from such an injury a permanent tinnitus aurium, this is explained by a partial, not complete, severance of the membrana tectoria from hair cells over a certain area. This alteration of the relation existing normally between hair cells and membrana tectoria may result, as we have repeatedly pointed out, in a stimulation of these cells. This explanation appears all the more rational since the pitch of the tinnitus is often approximately that of the whistle which originally produced the injury.

SUMMARY.

1. The hair cells of the organ of Corti are the real end organs wherein the physical impulses of sound waves are transformed into the nerve impulses which result in tone perception.
2. The perception for the various tones takes place in different parts of the cochlea, those of higher pitch being taken up by the hair cells located near the beginning of the basal coil, those of lower pitch by the cells near the apex of the cochlea.

3. The stimulation of the hair cells is effected only through the medium of their projecting hair.

4. The hypothesis that each hair cell acts as its own agent in selecting its stimulus from the impulses passing through the endolymph is shown to be untenable for a number of reasons, chiefly, however, because the relation existing normally between the hair cells and membrana tectoria will not permit of these impulses coming in direct contact with the hair cells. I have shown conclusively that the hairs of the hair cells project normally into the under surface of the membrana tectoria.

5. The stimulation of the hair cells is accomplished only through an interaction between the hairs of the hair cells and the membrana tectoria.

6. The hypothesis of Helmholtz that this stimulation is brought about through the vibration of the fibers of the membrana basilaris is untenable especially for the following reasons: In tracing the membrana basilaris toward the beginning of the basal coil in the vestibule this structure is found, at a considerable distance from the lower end of the coil and where a perfectly formed organ of Corti is still present to become so stiff and rigid as to render it incapable of vibrating. Even a complete absence of a basilar membrane in this locality is sometimes noted. The logical conclusion is that since the stimulation of the hair cells in this locality is accomplished without the intervention of a vibrating membrana basilaris, therefore, the stimulation of the hair cells throughout the cochlea is not dependent on the vibration of this membrane.

7. The logical conclusion is that the stimulation of the hair cells is accomplished through vibrations of the membrana tectoria transmitted to it by impulses passing through the endolymph.

8. The membrana tectoria is shown to be so constituted anatomically as to be capable of responding to the most delicate impulses passing through the endolymph. Furthermore, the great variation in size of this membrane from one end of the cochlea to the other, together with its lamellar structure, suggest the probable physical basis which renders it capable of acting the part of resonator by responding in one part to impulses of a certain pitch and in another part to impulses of another pitch.

9. The phenomenon of "beats" is more readily accounted for than by the Helmholtz hypothesis; moreover, all the scientific observations by Helmholtz and his followers supporting a resonator hypothesis apply more readily and with greater force to my theory, viz., that the delicate membrana tectoria acts as resonator.

10. Finally, the pathological phenomena of "tone islands," "dipla-

cousis binauralis dysharmonica," and of "tinnitus aurium" are all plausibly accounted for in this conception of the physiology of tone perception.

11. To restate briefly the process by which the phenomenon of tone perception is accomplished: The sound waves conducted from the air impinge upon the membrana tympani, producing vibrations in it. These vibrations conducted along the chain of ossicles transmit impulses to the intralabyrinthine fluid through the medium of the foot-plate of the stapes. The impulses originating in the fluid in the vestibule pass directly into the scala vestibuli and through the membrane of Reissner to the endolymph where sympathetic vibrations are imparted to the several parts of the membrana tectoria depending on the pitch of the tone. The vibrations of the membrana tectoria in turn stimulate the hairs of the hair cells which normally project into its under surface. The nerve impulses originating from all the hair cells thus stimulated by a particular tone come together in the brain center in the cortex when the tone picture forms the final step in the process of tone perception.

* * *

Since placing this paper in the hands of the publishers my attention has been called to an article by K. Kishi, which appeared in a recent number of Pflüger's Archives (Band CXVI, S. 112). Kishi expresses the view that the basilar membrane is not a vibrating structure. He has failed, as have others, apparently, to find what appears to me as a fundamental objection to this hypothesis, in that as the lower end of the basal coil is approached this structure not only becomes so stiff and rigid as to preclude the possibility of its being capable of vibrating but even its complete absence is sometimes noted. Kishi concurs in the view previously expressed by others, Siebenmann, for example, that the membrana tectoria appears to be the logical structure for applying stimulation to the hair cells. Just how the tectorial membrane accomplishes this function Kishi does not elucidate. His views regarding the character and relations of the membrana tectoria are quite at variance with my own findings, as shown in this article. He believes that this membrane is a taut structure attached to the labium vestibulare and to the outer end of the reticular membrane. The illustrations which Kishi publishes of a flattened-out tectorial membrane lying over the organ of Corti is not unlike others that appear in the literature and represent quite common findings, of which I have a great many. That such a membrane

represents a marked distortion is quite evident from the study of preparations such as I have shown in my drawings where the separate lamellæ can be traced running their entire course without so much as a fold or ruffle. The type of resonator which Kishi seems to have in mind for the membrana tectoria is that of a series of taut strings attached at either end. This is not in keeping with the anatomical facts as brought out in this paper.

ABBREVIATIONS USED.

- M. t.* = Membrana tectoria.
M. b. = Membrana basilaris.
L. v. = Labium vestibulare.
L. t. = Labium tympanicum.
L. s. = Ligamentum spirale.
C. l. s. = Crista ligamenti spiralis.
M. R. = Membrana Reissneri.
P. S. = Prominentia spiralis.
S. v. = Stria vascularis.
S. H. = Streifen of Hensen.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1. Section from lower end of basal coil showing membrana basilaris too thick to be capable of vibrating.

FIGS. 2, 6. Section from lower end of basal coil showing complete absence of a membrana basilaris. The organ of Corti rests on the firm structure of the crista of the spiral ligament which is attached directly to the labium tympanicum.

FIG. 3. Section similar to Fig. 2. A solid bony plate extending across from the outer wall of the cochlea to join the lamina spiralis ossea.

FIG. 4. Membrana tectoria attached to the organ of Corti. Streifen of Hensen partially withdrawn from its normal attachment to the supporting cells just internal to the inner row of hair cells. These supporting cells have also been somewhat pulled out. The preparation shows the inner hair cell detached from the membrana tectoria. The outer hair cells are still attached to the membrana tectoria.

FIG. 5. Membrana tectoria partly withdrawn from the organ of Corti. The Streifen of Hensen detached from its normal support as shown in Fig. 4. Inner hair cell free from membrana tectoria, the outer hair cells still clinging.

PLATE II.

FIGS. 6, 7, 8, 9, 10. A series of sections from a single labyrinth drawn to scale showing variation in size of membrana tectoria from the beginning of basal coil to apex of cochlea.

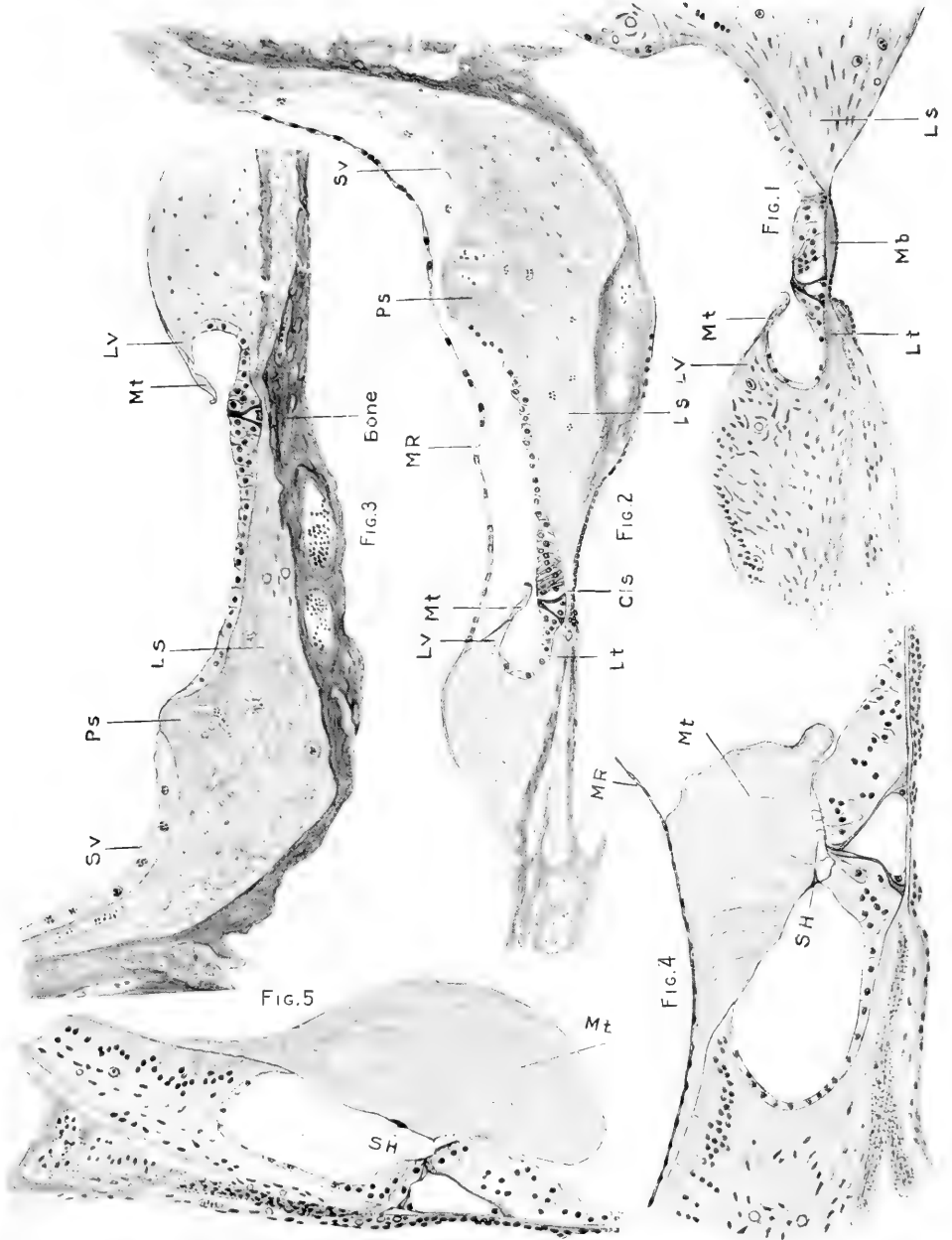






FIG. 6

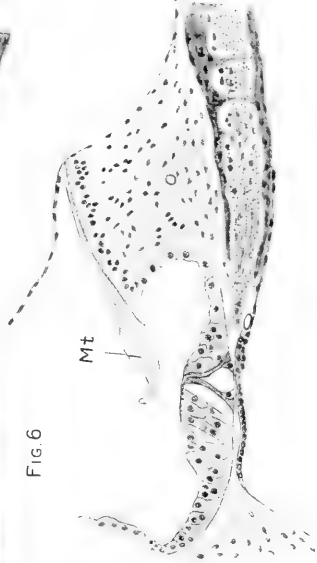


FIG. 7

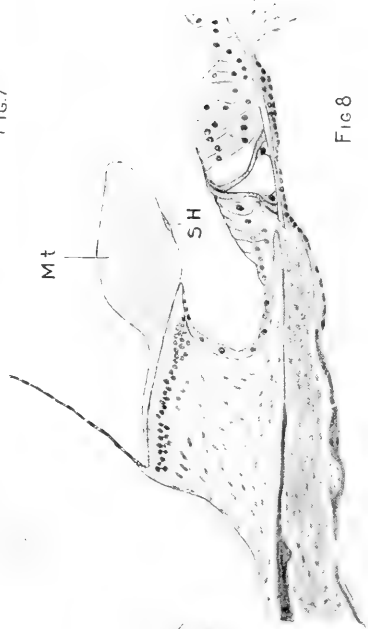


FIG. 8

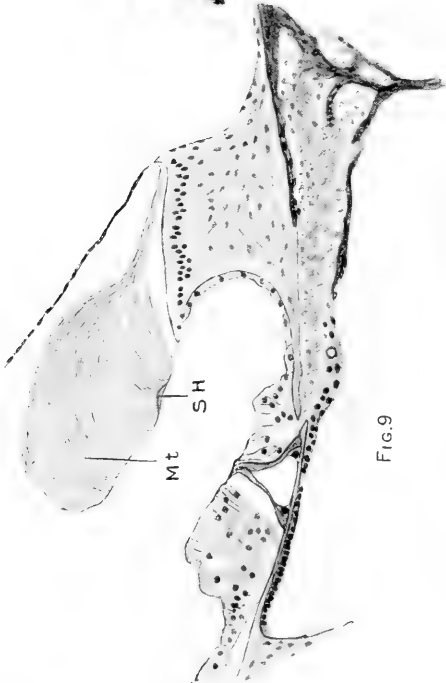


FIG. 9

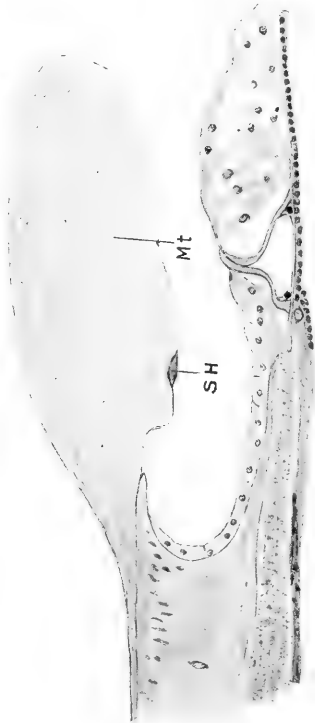


FIG. 10

EXPERIMENTS ON THE ORIGIN AND DIFFERENTIATION OF THE OPTIC VESICLE IN AMPHIBIA.

BY

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WITH 32 FIGURES.

ON THE SELF-DIFFERENTIATION OF THE OPTIC VESICLE.

In *rana palustris* at a time when the medullary plate first becomes visible, the tissue which later goes to form the eye possesses great power of self-differentiation. There is no indication at this time of any histological differentiation, in the eye region, between those cells destined to form eye or brain; nevertheless, if one transplants a small piece of the eye region from the anterior end of such a medullary plate into the mesenchyme of the otic region of a somewhat older embryo, the piece will continue to differentiate into eye and brain tissue, the various layers of the retina, including the pigment layer, will develop and invagination will take place. The normal environment then is no longer necessary for the continued differentiation of the eye at this period. I have only this one preliminary experiment at this stage and none at still earlier stages, so it is impossible to determine without further experiment at how early a stage the eye becomes self-differentiating.

My pupil, Mr. Fisher, has found that the eye rudiment at a later phase of the medullary-plate stage, in *rana sylvatica*, when transplanted into another embryo possesses the same remarkable power of self-differentiation. His work will soon be published.

The present paper is concerned, for the most part, with the differentiation and regeneration of the eye at a still later stage, at about the time of or shortly after the closure and beginning fusion of the neural folds. The optic vesicle at this time in both *rana palustris* and *rana sylvatica* has remarkable powers of self-differentiation. At this stage the optic vesicle projects from the side of the brain and produces a bulging of the ectoderm on the surface of the embryo.¹ The cavity of the optic vesicle

¹ Lewis, *Am. Jour. of Anat.*, Vol. VI, p. 493, 1907, Figs. 1, 2, 3, and 3a.

communicates with the brain cavity by a wide opening and there is very little indication of the formation of the optic stalk. The walls of the optic vesicle are of about the same thickness as the adjoining ventral wall of the brain. There is at this time no indication of any histological differentiation between those cells which are destined to form the eye or the optic stalk and the brain. That there are differences not brought out by the ordinary histological methods is evident from the results of the following experiments. A skin flap was turned back from over the optic vesicle without injury to it, and the eye was then cut off close to the brain and transplanted in most of the experiments beneath the ectoderm in various regions of the same or another embryo of the same age or even an older embryo. This is the same operation I have referred to in my papers on the origin of the lens.² It is impossible in working with such small objects to do each operation exactly alike, nor did I attempt especially to make the cut separating the optic vesicle from the brain in the same place each time. There were also slight variations in the ages of the embryos and in the extent of evagination of the optic vesicle.

These optic vesicles transplanted into various regions of the same or other embryos continue to differentiate in the new environment, sometimes at about the same rate as if they had remained in the normal position and attached to the brain. Sometimes, however, there was considerable retardation in the rate of differentiation of the various layers of the retina during the early stages, but in the older eyes no distinction can be noticed between the amount of differentiation of transplanted and normal eyes. Such transplanted eyes, whether in the otic region, the abdominal or tail regions of the same embryo or an older embryo of the same or another species continue to increase in size, to invaginate whether a lens is formed or not, and the pigment layer and various layers of the retina develop in a perfectly normal manner, provided the transplanted eye was not severely injured or distorted at the time of the operation. I have already figured a number of such transplanted eyes.³ It often happened that such transplanted eyes were distorted and torn during the operation and as a result many of them are more or less irregular in shape, especially as regards the cup cavity. But even in these distorted ones the various layers of the retina are present though

² Am. Jour. of Anat., Vol. III, p. 507, 1904; Vol. VI, p. 473, 1907; Vol. VII, p. 146, 1907.

³ Lewis, Am. Jour. of Anat., Vol. III, 1904, Figs. 20 and 22, p. 518, 24 and 25, p. 519, and 28, p. 521; *ibid.*, Vol. VII, 1907, pp. 159-169, Figs. 33, 34, 35, 37, 38, 40, 41, 42, 44, 47, 50, 52, 57, 61, 63, but especially Figs. 39, 42, and 44, p. 161.

distorted also. It is among these injured and distorted eyes, but more especially among the smaller transplanted eyes, that retardation in the differentiation of the retinal layers is to be observed during the early stages. When such eyes are transplanted into the same or even into the other species of rana they continue this progressive development for at least 20 days, which is as long as they were allowed to live. How much longer differentiation and growth would continue can only be determined by experiment. I have already shown that such transplanted eyes have the power of stimulating lens-formation from the strange ectoderm of the region into which they are transplanted.⁴ When in the proper relation with the ectoderm corneal changes will also take place in the ectoderm of the strange region. The anterior endothelial layer over the pupil of the eye will also form.⁵ All of these changes may take place except that of lens and corneal formation no matter what the orientation of the eye in the mesenchyme may be.

Such transplanted eyes as a rule have no connection or contact even with the central nervous system of the host, each eye being as a rule entirely surrounded by mesenchyme or at least becomes so a few days after transplantation. Such eyes as are in contact or become connected with the central nervous system or other structures of the host are apparently unmodified by such connection except by the fusion of the transplanted and the normal eye or the transplanted and regenerating eye.

If a portion of the transplanted eye projects into the cœlom a modification in the development of the pigment layer occurs in that the pigment layer fails to develop over this part of the eye. Fig. 7, from such an eye projecting directly into the cœlom, shows the pigment layer wanting here. Had the mesothelial lining of the cœlom been intact the pigment layer would probably have developed over this portion of the eye as it has over the transplanted eye seen in Fig. 8. Here the pigment layer is in contact with the mesothelial layer which prevents the eye from projecting into the cœlom.

It often happened that the cut separating the optic vesicle from the brain was in such a plane as to include a small amount of brain tissue, the latter remaining attached to one side of the optic vesicle. The resulting differentiation of the two tissues, after transplantation, into eye and brain tissue, may go on without the formation of an optic stalk, the

⁴ Lewis, *Am. Jour. of Anat.*, Vol. VII, 1907, p. 145; *ibid.*, Vol. III, 1904, p. 505.

⁵ Lewis, *Am. Jour. of Anat.*, Vol. VII, 1907, p. 161, Figs. 39, 41, and 42.

brain tissue remaining attached to the eye by its pigment layer. Or an optic stalk may differentiate with the brain tissue at one end and the eye at the other. Each tissue seems to continue its own line of development, apparently independently of the other, and no distinction is to be observed in invagination, or differentiation of the layers of the retina between those eyes with and those without attached brain tissue.

It has already been shown that small pieces of the rudiment of the central nervous system possess remarkable powers of self-differentiation.⁶ The optic vesicle and the brain then stand out in marked contrast to the lens and cornea in their power of self-differentiation. Le Cron⁷ has shown for *amblystoma* and I have shown in *rana palustris* and *rana sylvatica*⁸ that the lens is absolutely dependent on the continued influence of the optic vesicle for its origin, early growth, and differentiation. The cornea⁹ likewise lacks the power of self-differentiation and self-origination.

Among the transplanted eyes there are many very interesting variations, in size, shape, and differentiation, dependent for the most part upon the amount and distortion of transplantation. Fig. 1 shows a large, almost normal, transplanted eye, with quite regular invagination; a lens, endothelial layer, cornea, the pigment layer, and the various layers of the retina with the rods and cones are present. The ganglionic layer, however, is defective, as its cells are few and scattered, not forming a continuous layer as seen in the normal eye (Fig. 2). There are several such eyes among my experiments and in some of them an unusually large lens (in proportion to the size of the eye), which has retained its attachment to the entire inner layer of the retina even long after the usual time for the separation, may be accountable for the thinning out of the ganglionic layer. Some of the transplanted eyes show just the opposite condition and the ganglionic layer may be several cells thick and the other layers also thicker than normal. Such eyes show very little invagination or very small cup cavities, and as a rule no formation of a vitreous humor. And as in the eye of Fig. 5 the invagination may be of such a nature that the pupil is entirely obliterated and the cavity reduced to a minute space. In Fig. 5 the ganglionic layer is several cells in thickness and the outer pigment layer is unduly large in proportion to the size of the eye.

⁶ Lewis, *Am. Jour. of Anat.*, Vol. VII, 1907, p. 138.

⁷ *Am. Jour. of Anat.*, Vol. VI, 1907, p. 245.

⁸ Lewis, *Am. Jour. of Anat.*, Vol. VI, 1907, p. 473.

⁹ Lewis, *Jour. of Expt. Zool.*, Vol. II, 1905, p. 431.

Some of the transplanted eyes are very irregular in shape, owing to absence of or irregularity in the invagination, due to distortion in transplanting. Figs. 3 and 4 are from an eye where invagination failed to take place. Fig. 3 from a section very near one end of the eye shows only a small area of pigment layer, the larger part of the eye is entirely without a pigment layer and forms a flattened vesicle, the walls of which are composed of the various retinal layers with the rods and cones lining the cavity (Fig. 4). Figs. 8, 16, and 18 show very irregular eyes.¹⁰ In these the distortion of the vesicle at the time of transplantation was probably too great to be overcome by any regulative mechanism. In these as in Figs. 3 and 4 and in others the arrangement of the various layers of the retina is so irregular as to suggest the idea that at the time of transplantation there is already a determination of the various cells which go to form the several layers of the retina and they seem to continue along this predetermined path of differentiation in spite of the alteration of their position in the eye, so that those destined under normal conditions to form rods and cones, for example, do so in the distorted eyes and thus, as in Fig. 3, small groups of rods and cones *r. r.* are found separated off from the rest of the layer. In Fig. 29 a similar small group of rods and cones is seen in the center of the inner granular layer.¹¹

The small extent of the pigment layer in some eyes as in Figs. 3 and 4 or its excessive extent as in Fig. 5 suggest that at the time of the operation the cells which go to form the pigment layer are likewise already predetermined, and so, if but a few are transplanted with the eye this layer is scanty, while on the other hand, in some transplanted eyes, the proportion of cells destined to form pigment layer to those destined to form the other layers of the retina may be so large as to give rise to eyes with a great excess of the pigment layer as in Fig. 5. This predetermination in the fate of the cells seems to be borne out by the finding occasionally in the mesenchyme small detached groups of pigment cells of the same character as those in the eye. Such groups probably owe their origin to bits of detached optic vesicle which were broken away at the time of the operation. Small pieces of retina were also found (see Fig. 6), but here a small mass of pigmented cells is connected with it. Other pieces show only retinal cells without any pigment.

Under certain conditions invagination may fail entirely and the eye

¹⁰ See Figs. 2, 6, 8, 17, 19, 28, 30, 40, 41, 49, 55, 56, 57, and 59, Lewis, *Am. Jour. of Anat.*, Vol. VII, 1907, pp. 153-167.

¹¹ See also Fig. 44, Lewis, *Am. Jour. of Anat.*, Vol. VII, 1907, p. 163.

remain as a vesicle, connected with the ventricle of the brain by a wide opening as seen in Fig. 9. Here the various layers of the retina have developed, but the rods and cones and pigment layer are not in contact. The pigment layer does not possess, as in a normal eye, the numerous fine processes which run in between the rods and cones (compare Figs. 11 and 12). In Fig. 9 the section is directly through what would have been the cleft region, for we see here on the ventral side, the retina continuing without the intermission of the pigment layer directly into the optic stalk and here the optic nerve leaves the retina and enters the optic stalk. A neighboring section shows the fovea centralis, and still more lateral sections at one side or other of the cleft region show as in Fig. 10 a considerable extent of pigment layer on either side of the retina. This eye illustrates again the idea that there is not much of any correlation within the eye which determines the fate of the various cells of the optic vesicle, as for example, the outer pigment layer is not dependent for its differentiation on a contact relationship between it and the retina.

The choroidal fissure is present in a number of the transplanted eyes, both regular and irregular ones. In Fig. 17 it is cut at an angle and cannot be clearly shown in a section, but in studying the series its presence is evident. Fig. 16 is from a section directly through the fissure of this rather irregular eye. Continuous with this region and extending to the pigment layer is an area of what is evidently optic stalk tissue.

An optic stalk is plainly to be seen in a number of the eyes (see Figs. 13, 17, 18, 19, 20, 21, and 22). A piece of brain tissue may or may not be attached at the other end of the optic stalk. There is no brain tissue connected with the eye or optic stalk of Fig. 17 for example. On the other hand, the optic stalk may develop without an eye, as in Fig. 24. In this experiment the optic vesicle was cut away in such a manner as to leave no tissue for the regeneration of an eye and yet there is connected with the brain in the normal position a short, perfectly normal optic stalk. Fig. 25 shows another very similar example. The cut separating the eye from the brain in these experiments was probably in such a position as to leave no cells behind for regeneration of the eye; a few cells, however, were left attached to the brain which were destined to form optic stalk and have done so in the absence of the eye. In many of the transplanted eyes the optic stalk differentiates (see Figs. 13, 17, 18, 19, 20, 21, and 22) and becomes sharply distinguishable from the pigment layer to which it is usually attached. The presence or absence

of the brain tissue or of the retina has apparently no influence then on the differentiation of the optic stalk.

In these transplanted eyes the optic nerve appears to rise from the ganglionic layer and in some of the eyes passes through the choroidal fissure and into the optic stalk (see Figs. 17, 19, 20, 21, 22, and 23), to be traced in some specimens along the entire length of the optic stalk into an attached piece of transplanted brain, or the nerve may pass through the fissure and thence into the medulla of the host.¹² The nerve may penetrate the retina in various places other than the choroidal fissure. In one of the eyes it extended as far as the outer molecular layer, in which layer it continued for some distance as a distinct bundle. In a few of the eyes the nerve ends abruptly at the pigment layer and seems to have been unable to penetrate either into or through it (Fig. 30). In many of the transplanted eyes the nerve passes through the retina at one place or another into the pigment layer, it then continues in this layer as a distinct bundle of fibers between the pigment cells¹³ (Fig. 29). It may continue in this manner for long distances, one-half to three-fourths the circumference of the eye, and end abruptly here without leaving the eye.

The nerve may penetrate the pigment layer and run into the mesenchyme (Fig. 30) or may even enter the medulla.¹⁴ And even in the medulla the nerve can be traced as a distinct compact bundle through many sections (Fig. 31). The nerve may sometimes split as it passes through the retina into two or more separate bundles (Figs. 17 and 30). In a few instances the nerve instead of passing through the retina extends directly from the ganglionic layer across the cavity of the eye and through the pupil into the mesenchyme.¹⁵ Such variations in the optic nerve suggest at once that the axis cylinders are outgrowths from the cells of the ganglionic layer and that they follow the path of least resistance which, under normal conditions, would lead through the cleft and along the optic stalk into the brain. When the normal relations are disturbed, as in transplantation, the path of least resistance is disturbed with resulting variations in the course of the nerve.

Where the optic nerve passes directly from the ganglionic layer across the cavity of the eye it would seem as though some disturbance in the arrangement of the flattened ends of Müller's fibers caused an imperfect

¹² Lewis, *Am. Jour. of Anat.*, Vol. VI, 1907, p. 471, Figs. 14 and 15.

¹³ *Ibid.*, Figs. 20 and 21.

¹⁴ *Ibid.*, Figs. 14 and 15.

¹⁵ *Ibid.*, Figs. 20 and 21.

formation of the internal membrane-like structure and instead of directing the axis cylinders, as under normal conditions, towards the cleft and optic stalk they passed through the membrane into the cup cavity and so out through the pupil into the mesenchyme. By other disturbances in this membrane or by a change in the orientation of the ganglion cells we can account in a general way for the nerve penetrating the layers of the retina at various places, and by a variable path of least resistance the various paths of the nerves beyond the retina or in the pigment layer.

I should not wish to leave the impression that the eye possesses no, or very little, regulative power, but rather that a distortion in transplanting may readily be so extensive that a perfect eye cannot form.

Several eyes can probably develop from a single optic vesicle; Fig. 13 shows a large transplanted one, Fig. 15 a small transplanted one, and Fig. 14 the regenerating eye, all originally from the same optic vesicle.

The total volume of the transplanted and regenerated eye from the same original optic vesicle may be nearly twice as great as the normal one, indicating that both transplanted and regenerating eyes possess considerable power of regeneration.

ON THE REGENERATION OF THE EYE.

The right eye was cut away in 350 embryos, 180 of *rana palustris* and 170 of *rana sylvatica*, and the embryos were killed from 2 to 20 days after the operation. The optic vesicle was cut away, as we have already noted, at about the stage of closure of the neural folds and in most of the experiments transplanted into the same or another embryo.

The results as regards the regeneration of the eye from these operations are very varied but are all readily explained on the assumption, 1st, that those cells which go to form the eye are already determined and that the line of separation between brain and eye cells is fairly sharp and can be indicated by a plane having a position somewhat as the line *cd*, Fig. 3a.¹⁶ 2d, That at this stage regeneration of the eye can only take place from these cells which are already determined, and that the brain is not capable of regenerating an eye.

In a number of the embryos killed even as late as 20 days after the operation there is no trace of a regenerated eye. Some of these show a perfect bilateral symmetrical brain, and a transplanted eye often as large or larger than normal which may or may not have a small piece

¹⁶ Lewis, *Am. Jour. of Anat.*, Vol. VI, 1907, p. 493.

of brain tissue transplanted with it. The brain possesses considerable power of regeneration and when a small piece of brain tissue is cut away with the eye the lost part is often regenerated. In some experiments the brain may show some irregularity without any loss of tissue and the transplanted eye is usually large and without any transplanted brain tissue. Often, however, the brain is not only slightly irregular but evidently has suffered more or less loss of tissue, the latter having been cut away and transplanted with the eye; this defect and the amount transplanted may be very slight. In many experiments there is considerable asymmetry, showing more loss of brain than can be regenerated even after 20 days, and in most of these experiments there is associated with the transplanted eye a considerable piece of transplanted brain tissue. In none of the 350 experiments, however, was there very much brain tissue removed and never any from the other side of the head.

In some of the experiments the optic vesicle was cut in such a way as to leave behind cells enough to form the most proximal portion of the optic stalk, that part embedded in the brain.¹⁷ The brain in such cases may or may not be defective and the transplanted eye may or may not have brain tissue with it. In many of these experiments the defect in the brain is dorsal or anterior to the optic stalk showing that the piece of brain transplanted with the eye was from this region rather than the ventral optic stalk region. One of these embryos was killed as late as 19 days after the operation, yet nothing but the proximal portion of the optic stalk had regenerated.

In some of the embryos the optic stalk is longer and projects from the side of the brain, as in Figs. 24 and 25, but has no indication of an eye at the distal end. In others a very small knob is to be found at the end of the optic stalk consisting of retinal and pigment layer cells (Fig. 26). Figs. 14 and 27 show very small regenerating eyes. I have pictured regenerating eyes of various sizes in a former paper.¹⁸ Such eyes vary greatly in size and are often more or less defective in shape. The smaller ones show considerable retardation in differentiation and do not so often stimulate lens-formation.

As a rule the larger the regenerated eye the smaller the transplanted eye. In many instances, however, the regenerated eye may be two-thirds or three-fourths the diameter of the normal eye and transplanted eye

¹⁷ Lewis, *Am. Jour. of Anat.*, Vol. VI, p. 493, 1907, Fig. 4.

¹⁸ Lewis, *Am. Jour. of Anat.*, Vol. VI, 1907, pp. 495-509, Figs. 7, 8, 9, 10, 11, 12, 13, 14, 16, 18, 20, 22, 23, 25, 27, 28, 31, 33, 34, 36, 39, 42, 59, 60, 61, 62, 63, 64, 67, 69, 71, 72, 73, 74, and 76.

from the same optic vesicle lying in the same embryo just caudal to the regenerated one may be from two-thirds to seven-eighths the diameter of the normal one. Thus the regenerated and transplanted eyes may together be nearly twice as large as an eye the same optic vesicle tissue would have produced under normal conditions, and the cutting away a portion of it must have disturbed in some way the regulative mechanism.

We have already noted that in many of the experiments there is no regeneration of the eye and in others only regeneration of the optic stalk and in the remainder regenerated eyes of various sizes from extremely small ones to some nearly as large as normal. Amount of brain tissue cut away was never very extensive, and in some of the experiments there is no evidence of any excision of brain tissue, and this applies to all grades of the regenerated eyes, even in cases where there was no regeneration, and in no instance so far as I am aware was there any brain tissue taken from the opposite half of the brain. The age of the embryo within the limits of the time they were allowed to live after the operation, 2 to 20 days, does not influence these results.

Those experiments in which there was no regeneration or only regeneration of the optic stalk seem to me to indicate very clearly that the brain has no power of regenerating an eye. Bell (experimental studies on the development of the eye and nasal cavities in frog embryos,¹⁹ preliminary communication) makes the statement that "the retina may certainly be regenerated in very young embryos after removal of its entire anlage." He removed, after reflecting a skin flap, the anterior lateral one-half of the brain and optic vesicle of embryos of *Rana esculenta*. These embryos were from 2.5 mm. to 3.5 mm. in length, the tail bud had developed, the optic vesicle is present, and the lens is to be recognized as a slight thickening of the ectoderm,²⁰ if so he used a slightly older stage than I have used. At Bell's stage when the lens-bud is present the retinal portion of the optic vesicle is firmly adherent to it, in *Rana palustris*, *Rana sylvatica*, and *Amblystoma*, and this, I judge, must be the condition in *Rana esculenta* also. In *Rana palustris*, for example, it is very difficult and often impossible to reflect a skin flap from over the optic vesicle and not leave some of its cells attached to the skin flap, and I imagine in Bell's experiments some eye cells were left attached to the skin flap which was then replaced over the brain and pressed down. These cells thus devel-

¹⁹ *Anat. Anz.*, XXIX, 1906, p. 186.

²⁰ See his article in *Arch. f. Mikr. Anat. u. Entwicklungsgeschichte*, Bd. 68, 1906, pp. 279-280, Figs. 1 and 2.

oped into a retina, fused with the brain, and so give the appearance of a retina regenerating from the brain.

We have noted that the regenerating eyes vary greatly in size, this is dependent in part upon the age of the embryo, but to a greater extent, I believe, on the amount of optic vesicle tissue left attached to the brain, the smaller the amount left the smaller the eye, the greater the amount left the larger the regenerating eye.

In most of the eyes the retina, the pigment layer, and the optic stalk are found and usually in normal proportions. How does this happen that so many of the regenerating eyes show such normal proportions of these tissues? It is scarcely probable that in cutting the eye away there was left each time just the right proportion of retinal, pigment, and optic stalk-forming cells. The explanation I have to offer is that some, enough at least of each of these kinds of cells were left and that owing to a self-regulating mechanism regeneration of each of these tissues, went on at rates in inverse proportion to the number of the cells of each left behind attached to the brain. A like explanation would be given also for the final formation of the transplanted eyes. According to this idea of inverse proportional regeneration, if the stump of an optic vesicle contained a large number of retinal-forming cells and a very small number of those destined to form pigment layer there would be a greater regenerative activity of the pigment-forming cells until they, by multiplication, were present in about the same proportion as in a normal eye. In some of the eyes this disproportion of the cells of various kinds may be so great that a normal eye cannot form. The question naturally arises as to whether optic stalk-forming cells might not form retinal or pigment-layer cells, or retinal cells, pigment cells, and vice versa.

The fact that after the optic vesicle is cut away the brain may show no indication of injury yet no indication of a regeneration of an eye or an optic stalk even be found, the fact that we get regenerating eyes of all sizes even days after the operations, the fact that among the transplanted eyes the pieces of brain tissue which were transplanted with them differentiate into brain tissue and are not incorporated as a part of the eye, would seem strong confirmation that the tissue of the optic vesicle and adjoining regions of the brain is already predetermined at the time of the operation and that brain tissue does not change into eye tissue or eye tissue into brain tissue and that the brain cannot regenerate an eye.

EFFECTS OF CROSS TRANSPLANTATION ON THE GROWTH AND DIFFERENTIATION OF THE OPTIC VESICLE.

Optic vesicles from *rana sylvatica* were transplanted into the region between the eye and ear of *amblystoma* embryos of various ages, namely, from a time before there are any traces of lens-formation until the lens-bud is well developed and nearly ready to pinch off. Fifty experiments were made and the embryos allowed to live from 2 to 27 days after the operation. The majority were killed 3, 4, and 5 days after transplantation. In most of these the transplanted optic vesicle goes on differentiating and growing in a very normal manner, invagination may take place and in 3 or 4 days the outer pigment layer is well differentiated. In the 5 days the invagination has progressed and not only the pigment layer but the other layers of the retina are clearly to be distinguished (Figs. 27 and 28), and in some of the embryos the optic nerves can be seen passing through the retina to the outer layer or into the mesenchyme. Not only is the optic vesicle under such conditions capable of self-differentiation and unaffected apparently by its environment, but it is probably capable of stimulating lens-formation from the ectoderm of the *amblystoma*? This will be more fully considered in a later paper. In some of the embryos the anterior endothelial layer formed from the mesenchyme of the *amblystoma* (Fig. 28) extends over the pupil and lens.

Unfortunately, all but 4 of the embryos were killed during the first 6 or 7 days, but one, however, was killed 17 days, two 19 days, and one 27 days after the operation. In the ones killed 17 and 19 days after the operation the cells of the pigment layer have undergone a most curious change, they no longer form a continuous layer about the retina but are scattered in the mesenchyme about the eye as large heavily pigmented cells. There are some indications of disintegration of the rest of the eye, yet, for the most part, the retina with its various layers is quite normal in appearance.

In the embryo killed 27 days after the operation only a few scattered cells were to be found. Unfortunately, this is the only embryo allowed to live so long and more experiments will be necessary before a final statement can be made of the ultimate effect on the transplanted eye of the surrounding environment. One of my pupils is now at work upon this question.

Twenty-five similar experiments were made of transplanting the optic vesicles of *rana palustris* embryos to the preotic region of *amblystoma* embryos of various stages. The results are identical with those of *rana*

sylvatica. The transplanted optic vesicle invaginates and the various layers of the retina form. Most of the embryos were killed from 3 to 6 days after the operation. In one allowed to live 17 days the pigment layer has broken down and its deeply pigmented cells are migrating into the mesenchyme. The various layers of the retina appear to show beginning disintegration.

Lenses are associated with several of these transplanted eyes but as none of them are attached to the ectoderm one is not absolutely sure of their origin, but I believe that here, too, the optic vesicle of *rana palustris* can stimulate lens-formation from the ectoderm of *amblystoma*. The normal lenses associated with the eye of the *amblystoma* embryos are present and uninfluenced by the formation of other lenses.

In six experiments the optic vesicles of *rana sylvatica* embryos were transplanted into the otic region of slightly older embryos of *rana palustris*. Such transplanted eyes go on differentiating and invaginating. In one experiment the embryo was killed 17 days after the operation and the transplanted eye, though very irregular in form, owing to distortion of transplantation, shows perfect differentiation of the layers of the retina and the pigment layer, and the latter in marked contrast to those transplanted into *amblystoma*, forms a continuous layer and shows no tendency towards migration of its cells. In three of the experiments lenses are associated with the transplanted eyes, in one a lens-like bud is still attached to the ectoderm by a long pedicle showing that such transplanted eyes have the power of stimulating lens-formation from ectoderm of another species.

The study of the reactions in the cross transplantation of tissues of various kinds into species as nearly related as *rana palustris* and *rana sylvatica* or as widely separated as *rana* and *amblystoma* offers a wide and interesting field of investigation.

FIG. 1. Experiment DF₉₂. Embryo *rana palustris* killed 17 days after transplantation of optic vesicle anterior to otic vesicle. Section through transplanted eye and lens. The ganglionic layer of the retina contains only a few scattered cells, otherwise the retina is quite normal, as in Fig. 2. No optic nerve was found. $\times 100$ diameters.

FIG. 2. Experiment DF₉₂. Section through part of normal retina, ganglion layer a continuous layer one cell thick. $\times 100$ diameters.

FIG. 3. Experiment DF₉₁. Embryo *rana palustris* killed 19 days after transplanting optic vesicle ventral in the otic vesicle. Section through caudal end of eye showing very small extent of pigment layer and irregular arrangement of retina. *r, r*, small areas of rods and cones. *m, m, m*, outer molecular layer. $\times 100$ diameters.

FIG. 4. Experiment DF₉₁. Section showing irregular arrangement of above transplanted eye. No pigment layer. Rods and cones project towards a central cavity in which are a few masses of pigment. All the layers of the retina can be distinguished but irregularly distributed. $\times 100$ diameters.

FIG. 5. Experiment DF₉₁. Embryo killed 7 days after transplantation of the eye anterior to the otic vesicle. Section through transplanted eye. The pigment layer entirely surrounds the eye and dips down in the region of the pupil, which is entirely obliterated. The cup cavity is very small. Between the outer layer and the forming endothelial layer is a small abortive lens. The various layers of the retina are thicker than normal. $\times 100$ diameters.

FIG. 6. Experiment DD₁₂. Embryo killed 4 days after transplantation of the eye. Section through small piece broken away from the eye during the operation showing retinal and pigment cells. $\times 200$ diameters.

FIG. 7. Experiment DL₉₈. Embryo killed 5 days after transplantation of the eye caudal to otic region. Section through transplanted eye and lens. The eye is median to anterior end of Wolffian body and the ventral portion of it projects into the coelom. The outer pigment is here wanting and the mesothelial layer of the peritoneum also, so that the small rods and cones project directly into the coelom. \times about 200 diameters.

FIG. 8. Experiment IV₁₁. Embryo killed 5 days after transplantation of optic vesicle caudal to otic vesicle. Section through irregular transplanted eye showing contact of pigment layer with the peritoneum (*p*) and also contact of the eye with the ectoderm (*e*). $\times 100$ diameters.

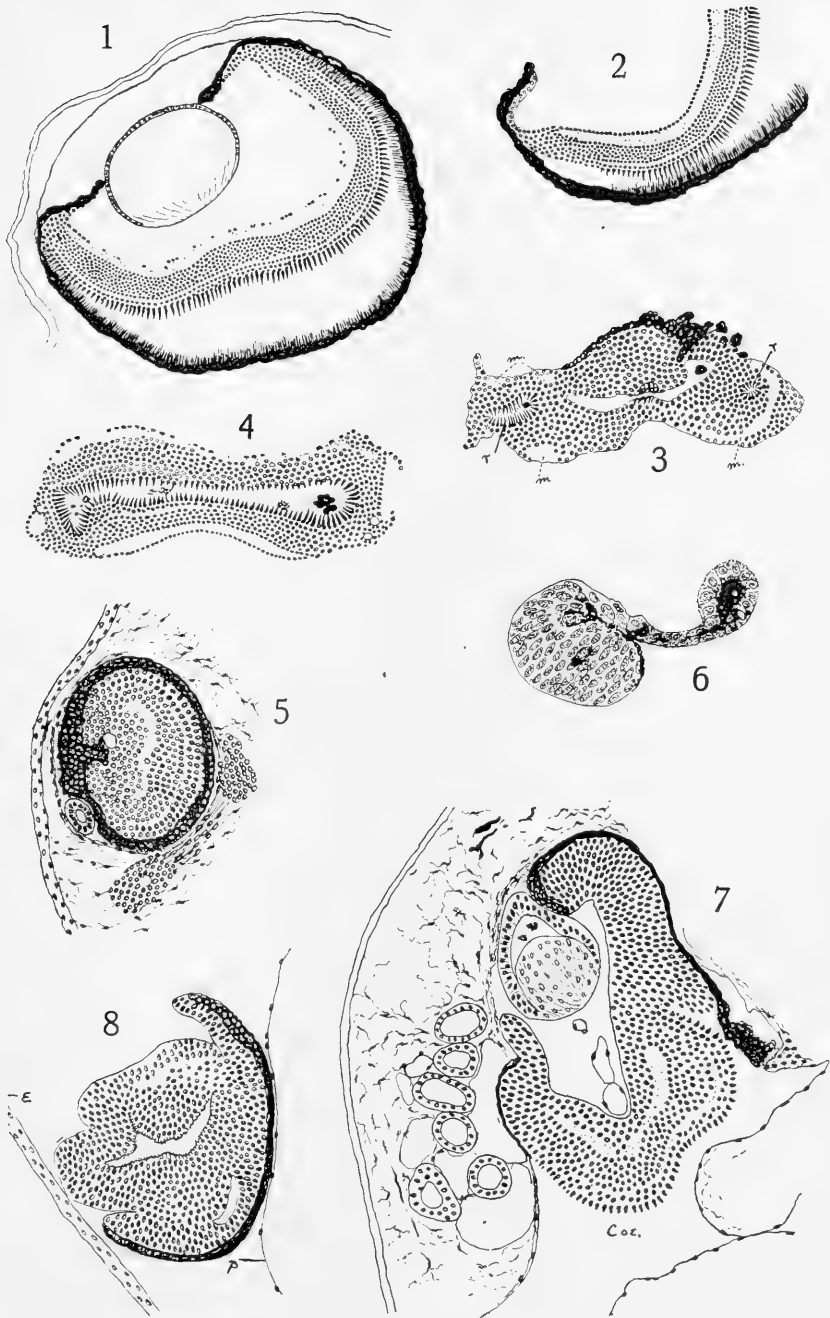


FIG. 9. Experiment XIII₃₆. The ectoderm over the right eye of a rana palustris embryo at the time of fusion of the neural folds was cut away and the caudal one-half of a somewhat older embryo grafted on so that the optic vesicle projected towards the cœlom. Embryo killed 17 days after. Section shows projecting from the side of the brain a large eye, which is in contact with the peritoneum by the retinal layer. Invagination did not occur and the large vesicle cavity is connected with the ventricle by a wide opening. $\times 45$ diameters.

FIG. 10. More lateral section through above eye. $\times 45$ diameters.

FIG. 11. Section through pigment layer of above eye. $\times 180$ diameters.

FIG. 12. Section through pigment layer of a normal eye. $\times 180$ diameters.

FIG. 13. Experiment DL₂₃. Optic vesicle of embryo of rana sylvatica was transplanted to otic region of same embryo. Embryo killed 4 days after operation. In transplanting the eye it was broken into two separate pieces. Section through larger piece of transplanted eye which lies ventral to the otic vesicle. This transplanted eye shows regular invagination and beginning differentiation of the retinal layers; a small piece of brain is attached to it. $\times 90$ diameters.

FIG. 14. Experiment DL₃₁. Section through small deeply situated regenerated eye. $\times 90$ diameters.

FIG. 15. Experiment DL₂₃. Section through part of transplanted eye which is entirely separate from regenerated or larger transplanted eye. $\times 90$ diameters.

FIG. 16. Experiment DF₄₇. Embryo killed 4 days after optic vesicle was transplanted just anterior to otic vesicle. Section shows irregular invagination in the region of the choroidal fissure. The optic nerve pierces the retina in a different place than the choroidal fissure. $\times 100$ diameters.

FIG. 17. Experiment DL₄₅. Embryo killed 4 days after transplantation of optic vesicle into the otic region. Section through transplanted eye and lens showing optic nerve and part of optic stalk into which part of optic nerve runs. $\times 100$ diameters.

FIG. 18. Experiment IV₉. Section through irregular transplanted eye of an embryo of rana palustris killed 5 days after the operation, showing differentiation of the optic stalk and irregular eye. $\times 100$ diameters.

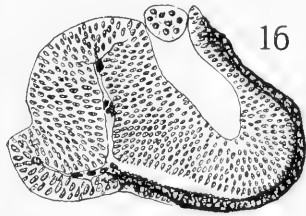
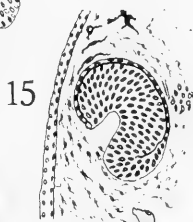
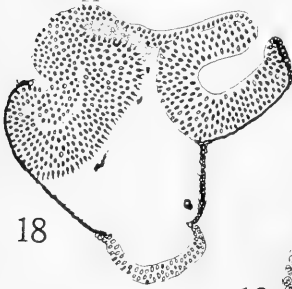
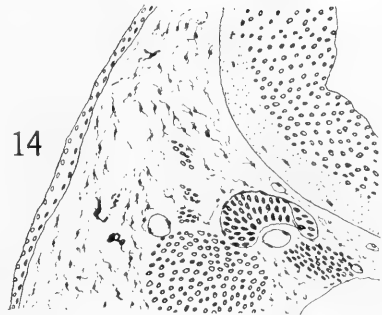
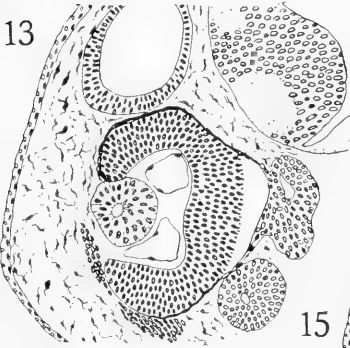
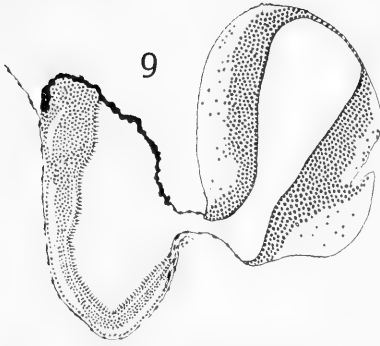
FIG. 19. Experiment DL₇₁. Embryo killed 5 days after transplantation of the eye ventral to the otic vesicle. Section through its optic stalk showing nerve fiber. $\times 200$ diameters.

FIG. 20. Section showing attachment of optic stalk to eye. The nerve fibers pass along the optic stalk to a piece of attached brain tissue which was transplanted with the eye. $\times 200$ diameters.

FIG. 21. Experiment DL₆₈. Embryo killed 4 days after transplantation of the optic vesicle into the otic region of the same embryo. Section through pigment layer and beginning of the optic stalk, showing position of nerve fibers at (o). $\times 200$ diameters.

FIG. 22. Section through middle of above optic stalk showing position of nerve fibers. $\times 200$ diameters.

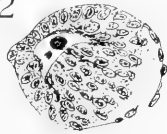
FIG. 23. Section through attachment of above optic stalk to a piece of brain which was transplanted with the eye, the optic nerve is seen passing from the optic stalk into the brain. $\times 200$ diameters.



18



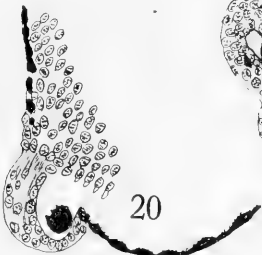
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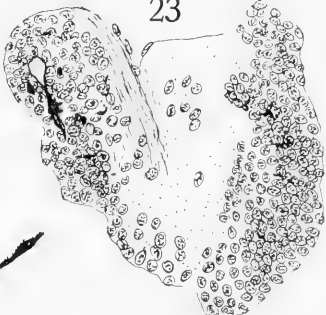
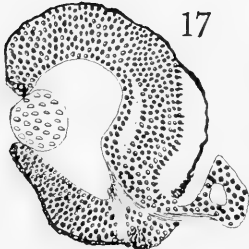


FIG. 24. Experiment DL₅₅. Embryo killed 5 days after cutting off and transplanting the right optic vesicle. Section through ventral portion of brain showing symmetrical position of two optic stalks. The left one (right in figure) extends to the normal eye, the other one extends but a short distance and ends abruptly. The transplanted eye is large and has a bit of transplanted brain attached to it. $\times 100$ diameters.

FIG. 25. Experiment DF₁₃. Embryo killed 4 days after the operation. Section through ventral part of brain and optic stalk. The normal left one reaches to the eye. The right one ends in a slight enlargement but has no pigment or retinal layer cells. The transplanted eye is large and there is no transplanted brain tissue. $\times 100$ diameters.

FIG. 26. Experiment dya₃. Embryo killed 9 days after cutting off the optic vesicle. Section through very small regenerated eye which has all the layers of the retina somewhat irregularly arranged. $\times 100$ diameters.

FIG. 27. Experiment IV₁₁. Embryo killed 5 days after cutting off the optic vesicle. There is a very small deeply situated regenerated eye with retinal and pigment layers, the differentiation of the retina is much retarded. The eye is connected with the brain by a large optic stalk. $\times 100$ diameters.

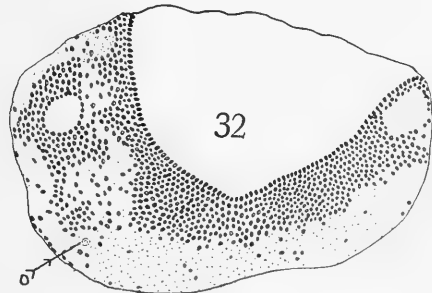
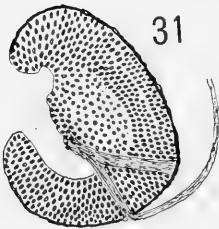
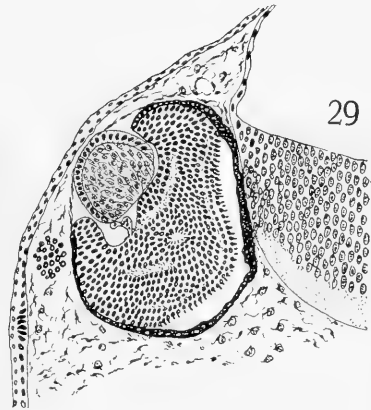
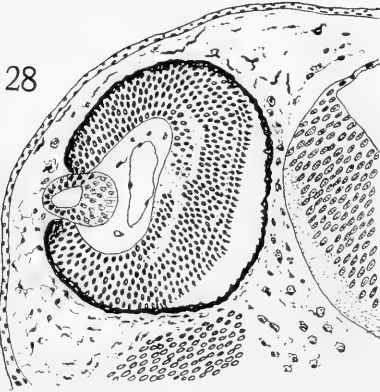
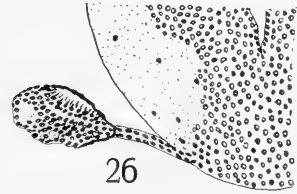
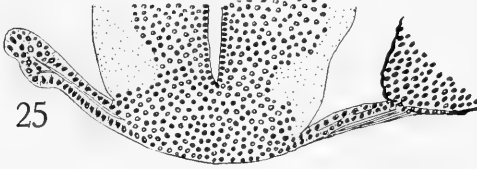
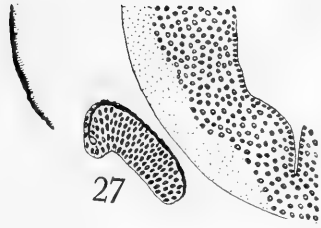
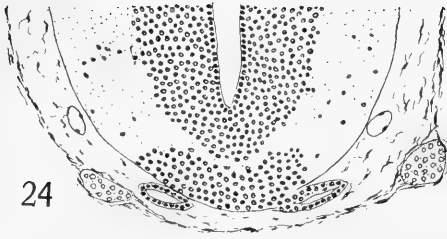
FIG. 28. Experiment DO₈. Optic vesicle of *rana sylvatica* at stage of fusion of medullary fold transplanted anterior to otic vesicle of amblystoma at stage when the lens-plate is quite thick. Embryo of amblystoma killed 5 days after the operation. Section through transplanted eye and lens and part of medulla showing large regular invagination of optic cup with formation of layers of the retina. $\times 90$ diameters.

FIG. 29. Experiment DO₃. Operation as in Fig. 28. Embryo of amblystoma killed 5 days after the operation. Section through transplanted eye and lens and part of medulla showing differentiation of eye and lens. $\times 90$ diameters.

FIG. 30. Experiment DF₁₂. Embryo *rana palustris* killed 4 days after transplantation of the optic vesicle into the region between medulla and otic vesicle. Section showing the relation of the optic nerve to the outer layer. The transplanted eye is without a lens but shows about the same degree of differentiation as the normal eye, the invagination cavity is shallow and the pupil wide. The optic nerve leaves the retina by the cleft region and passes into the outer layer. It runs in among the pigment cells of the outer layer as a distinct bundle for about $\frac{1}{2}$ the circumference of the eye. The nerve does not leave the outer layer, but remains as a compact bundle of fibers.

FIG. 31. Experiment DF₇₀. Embryo *rana palustris* killed 5 days after transplantation of the optic vesicle into the preotic region. Section through transplanted eye showing somewhat irregular invagination and the optic nerve. The latter has been projected in part into the figure from the neighboring sections. The optic nerve soon after its origin splits into two parts, the larger division runs through the retina to the outer layer but does not penetrate into it, its fibers ending among the rods and cones which are just developing. The smaller division enters the outer layer, runs for a short distance within it, then splits the smaller division ends in among the cells of the outer layer after running through a few sections. The larger division soon leaves the outer layer and runs for some distance in the mesenchyme and seems to join the third cranial nerve. There is no indication of a choroidal fissure in the transplanted eye. $\times 90$ diameters.

FIG. 32. Experiment DF₆₇. Optic vesicle transplanted caudal to the otic vesicle, its nerve passes into the medulla and can be traced anteriorly for many sections as a distinct bundle. Section through this bundle *o*. $\times 90$ diameters.



I. A STUDY OF CHROMOSOMES IN THE SPERMATOGENESIS OF ANASA TRISTIS.¹

BY

KATHARINE FOOT AND E. C. STROBELL.

WITH 3 PLATES AND 4 TEXT FIGURES.

Valuable contributions to our knowledge of the spermatogenesis of the Hemiptera have been made in recent years by Paulmier, 99; Montgomery, 01, 06; Gross, 04, and Wilson, 05-06, and Professor Wilson has recently created a new interest in this branch of cytology by contributing the authority of his support to McClung's hypothesis that in the definite distribution of a given chromosome we may have a morphological explanation of the determination of sex. Though Professor Wilson differs from McClung in some important particulars, they both agree as to the dimorphism of the spermatozoa, but McClung, 02, claims that spermatozoa (Orthoptera) *possessing* one extra chromosome produce males, while Wilson claims that spermatozoa (Hemiptera) *lacking* such an extra chromosome produce males.

Among the investigators of the spermatogenesis of the Insecta there is a striking agreement as to the fact of the dimorphism of the spermatozoa, most of them maintaining that half the spermatozoa receive one more chromosome than the other half, though there is an equally striking disagreement as to the time this unequal distribution of the chromatin takes place. It is held that in some forms this unequal distribution is caused by the omission of the division of one of the chromosomes of the first spindle and in other forms by the omission of the division of one of the chromosomes of the second spindle. This difference is not definitely associated with a given order. In the Hemiptera, for example, Professor Wilson finds eight forms in which the division of one of the chromosomes is omitted in the second spindle and two forms in which it is omitted in the first spindle, and Miss Stevens, 06, finds a similar inconsistency in Aphrophora.

In the spermatogenesis of *Anasa tristis* there are notable contradic-

¹We are indebted to the courtesy of Dr. P. R. Uhler for identifying our material.

tions in the original observations and interpretations of the investigators who have studied this form, Paulmier, 99; Montgomery, 01; Wilson, 05-06. Paulmier observed twenty-two spermatogonial chromosomes and this count was corroborated by Montgomery in 1901. Paulmier further observed the persistence of the two small spermatogonial chromosomes in the resting spermatocyte, the subsequent unequal distribution of these two chromosomes producing the dimorphism of the spermatozoa. According to Paulmier, these small chromosomes form a bivalent which divides in the first spindle but fails to divide in the second—*it passes undivided to one of the daughter cells*. In 1901 Montgomery confirmed these observations.²

A contradiction of these observations and interpretations was published by Professor Wilson in 1905. First, he finds twenty-one not twenty-two spermatogonial chromosomes. Second, the two small spermatogonial chromosomes named by Wilson, the "microchromosomes," do not persist in the resting spermatocyte. Third, the univalent microchromosome of the second spindle divides and, therefore, does not cause the dimorphism of the spermatozoa. Although Wilson accepts Paulmier's and Montgomery's conclusion as to the dimorphism of the spermatozoa, this he claims is due to the unequal distribution of one of the larger chromosomes. This chromosome he identifies as an odd spermatogonial chromosome, finding only twenty-one chromosomes in the spermatogonia. He observes the persistence of this odd chromosome during the rest stage of the spermatocytes, sees it divide in the first spindle, but in the second spindle pass undivided to one of the daughter cells. In a later work Montgomery, 06, withdraws his earlier indorsement of Paulmier's observations and unqualifiedly confirms Wilson in the important points in which Wilson differs from Paulmier, viz., the odd number of spermatogonial chromosomes (twenty-one instead of twenty-two) and the identification of this odd chromosome as the one which in the second spindle passes undivided to one cell, causing dimorphism of the resulting spermatids. Professor Wilson, 07, states that Paulmier, himself, admitted his error in the count of the spermatogonial chromosomes, though this does not necessarily imply an agreement with Professor Wilson's interpretation as to the significance of this odd chromosome. In any case, at present, these three investigators agree as to the odd number of spermatogonial chromosomes and the dimorphism of the spermatids and it is on these two points we take issue with them.

²"I am able to confirm Paulmier's account of the two naturation divisions," p. 168.

This dimorphism of the spermatozoa first observed in *Pyrrocoris* by Henking, 91, has received such wide support from later observers in a large variety of forms that it is with much hesitation we question its occurrence in a form so well known as *Anasa*. Realizing the futility of disputing the interpretations of the noted investigators of this form with the inadequate demonstration furnished by drawings, we have illustrated our evidence with one hundred and six photographs of our preparations—these being selected from a series of three hundred and thirty—these in turn being chosen from nearly a thousand cells indexed as sufficiently clear to be photographed. These examples were selected from the cells of more than fifty smear preparations. We have studied also sections of the testes and made a few photographs of these sectioned cells in addition to those above mentioned, but the photographs of Plates I to III are of our smear preparations. This data covers a relatively limited field of the development, *i. e.*, the spermatogonial chromosomes and from the growth period of the first spermatocyte to the telophase of the second spindle inclusive. These stages have been shown by Montgomery and Wilson in a few camera lucida drawings and this evidence we will later compare in detail with our photographs. In their investigations, however, they have covered a much broader field, having studied a large number of forms. We fully appreciate some of the advantages claimed for a broad comparative method of work, though we can scarcely agree with Montgomery, 06, when he says (in reference to another point under discussion), “no one has a right to express doubt . . . who has not made broad comparative observations of his own,” p. 152. In this connection it is interesting to question just how far we may safely follow a broad comparative method without sacrificing that painstaking attention to details acknowledged as indispensable in all lines of scientific work.

We believe that our preparations demonstrate the following points: First, that there are twenty-two spermatogonial chromosomes.

Second, that *none* of these chromosomes retain their morphological individuality throughout the growth period—neither the microchromosomes as claimed by Paulmier and Montgomery, nor an odd chromosome identified by Wilson, and later by Montgomery, as a heterotropic chromosome.

Third, that in the early prophase of our preparations the so-called odd (heterotropic) chromosome of Wilson and Montgomery (*i. e.*, the eccentric chromosome of the later prophases, or metaphase), resembles in no

way a nucleolus, and is morphologically wholly unlike the same chromosome figured by Wilson at this stage.

Fourth, that the eleven chromosomes of the first spindle are *all* bivalents and that the eleven chromosomes of the second spindle are *all* univalents.

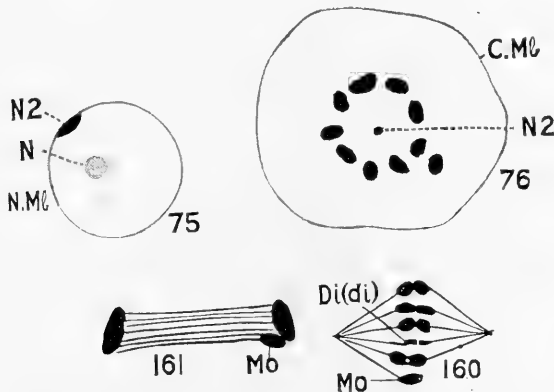
Fifth, that in *both* the first and second spindles one chromosome—which we believe to be the eccentric chromosome of the late first prophase—often lags in division, but that normally its final division occurs in both spindles.

Professor Wilson in his recent paper in *Science*, February, 07, replying to our preliminary note says, that he thinks the contradiction in our results is probably due to the difference of method employed, we having placed our faith in smear preparations while he has relied on sections. We are glad of an opportunity to emphasize this faith, believing that for demonstration of the structure and count of chromosomes our modified smear preparations are more reliable than sections, and it is for this reason we have abandoned the use of sections in studying chromosomes except for comparative work and for studying the topographical relations of the cells. In cells fixed and sectioned nearly all the delicate details shown in the chromosomes of our smear preparations are completely lost, and it ought to be too obvious to mention that a method which presents clearly each individual chromosome in its integrity offers decided advantages when the question of accurate counting assumes the importance and develops the contradictions familiar in recent literature. In spite of Professor Wilson's maintaining "that the determination of the number of chromosomes in a given species demands more critical treatment than the mere matter of counting or photographing," this elementary pursuit of counting chromosomes has certainly played a very significant part in recent observations.

*The Eccentric Chromosome, i. e., Accessory Chromosome, McClung, Odd or Heterotropic Chromosome Wilson, 05-06, Monosome Montgomery, 06.*³—The three investigators of the spermatogenesis of *Anasa tristis*

³As the names given to this chromosome in *Anasa* are each descriptive of the investigators interpretation we were unable to adopt any of them and in our preliminary paper we suggested "eccentric" as a convenient term. "The frequent eccentric position of this bivalent chromosome outside the characteristic ring arrangement of the chromosomes in the late prophase, seems to warrant suggesting "eccentric" chromosome as a convenient descriptive name for this special chromosome." Foot and Strobell, *Bio. Bull.*, Vol. XII, 1907.

agree as to the presence in the resting first spermatocyte of a deeply-staining nucleolar-like body and they all agree further that this body is present *in addition* to a plasmosome and it is, therefore, not a true nucleolus, but one (or two) of the spermatogonial chromosomes. Paulmier and Montgomery in their original work on this form identified this body—the chromosome nucleolus—as the two microchromosomes of the later stages. This interpretation is clearly represented in two of Montgomery's figures (Text Fig. 1). Here we see the N^2 (Fig. 75) of the resting spermatocyte identified as the N^2 (Fig. 76), microchromosomes of the first metaphase. In his later work (o6), Montgomery corrects

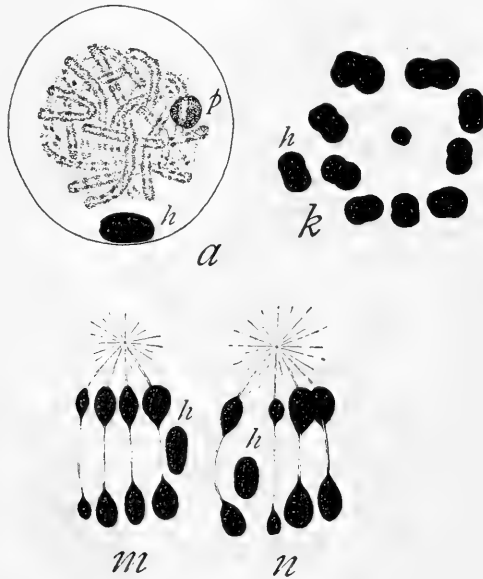


TEXT FIG. 1. Reproductions of four of Montgomery's sketches of *Anasa tristis*. Figs. 75-76, o5, and Figs. 160-161, o6. Fig. 75, Nucleus of first spermatocyte; Fig. 76, Pole view of monaster, first maturation mitosis; *C. Mb.*, cell membrane; *N.*, true nucleolus (plasmosome); *N.2*, chromatin nucleolus (accessory chromosome); Figs. 160 and 161, Second maturation spindles.

this interpretation, agreeing with Wilson in identifying this deeply-staining structure N^2 of the rest stage with the larger chromosome lying outside the circle of chromosomes shown in his Fig. 76 (Text Fig. 1). Montgomery's Fig. 75 (Text Fig. 1) and Wilson's Fig. *a* (Text Fig. 2) demonstrate that the structure which they interpret as an odd univalent chromosome (*h* of Wilson's figure) is a deeply-staining nucleolar-like body.

In our preparations the presence of such a deeply-staining nucleolar-like body in the nucleus of the first spermatocyte is demonstrated in Photos. 1 to 15, Plate I, but these photographs further demonstrate that in our preparations there is only *one* deeply-staining nucleolar-like body

present, and we must, therefore, interpret this body either as the chromosome-nucleolus of Wilson and Montgomery, or as a true plasmosome. As the presence of a plasmosome in the nucleus at these stages is the typical phenomenon familiar in all known forms (its absence being most exceptional) we feel justified in interpreting the structure we find in the resting nucleus as a true plasmosome and not an odd persisting spermatogonial chromosome, *i. e.*, chromosome nucleolus.



TEXT FIG. 2. Reproductions of three of Wilson's sketches of *Anasa tristis*, 05. *a*, Contraction-phase of synaptic period, showing "accessory" (*h*) and plasmosome (*p*); *k*, polar view of metaphase group, first division, *m*, *n*, anaphases of second division, showing division of *m*-chromosomes and the undivided heterotropic chromosome.

In our smear preparations we do not find *two* nucleolar structures of equal or nearly equal size such as Montgomery (Fig. 75, Text Fig. 1) and Wilson (*a*, Text Fig. 2) find in their sections at this stage.

As the chromosomes begin to form we often find, in addition to the persisting plasmosome, parts of one or more of the chromosomes or entire chromosomes condensed into a nucleolar-like mass, but we cannot interpret this as the normal condition. Such an abnormal appearance of several of the chromosomes is shown in Photo. 13, Plate I. The persisting plasmosome is present and parts of at least four of the chromosomes show evidence of this abnormal nucleolar-like condensation. This

condition is again strikingly shown in Photo. 18, Plate I. All the eleven chromosomes are clearly defined and in the center of the group two of the cross-shaped chromosomes are in contact and between them a densely-staining mass of chromatic substance which, though resembling a nucleolus, we interpret as an abnormal condition of the two arms of the crosses which are in contact. In Photo. 1, Plate II, we see one arm of the large cross-shaped chromosome showing a like abnormal condition, and such a condensation of the chromatic substance of one or more of the chromosomes is not confined to the prophases of the first spindle, it may occur at any stage of both divisions.

The stage at which Wilson so clearly differentiates two nucleolar-like structures (Text Fig. 2*a*) is probably the same stage we show in Photo. 11, Plate I. This photograph shows the complete absence of a second nucleolar-like structure, and we would accentuate here the advantages of our smear preparations—an *entire nucleus* is dried on the slide and it cannot, therefore, be claimed that any of the structures may be cut off as in the case of sections. At this stage, however, we often find *in sections* two nucleolar-like structures, but there are three facts to be considered in this connection: First, we do not find more than one such nucleolar-like body in the resting spermatocyte of our smear preparations, and second, we sometimes find in sections not only two but three, four, or even five nucleolar-like bodies. In those cases in which three or more are seen, we are forced to interpret these bodies as probably representing the abnormal condition of the chromatin or chromosomes described above for the preparations of Photos. 13 and 18, Plate I, and Photo. 1, Plate II, and this makes us very cautious in attributing much significance to the sections in which we find only two. And, finally, it cannot be claimed that the elongate form often figured for the chromosome nucleolus in the rest stage is absent in our smear preparations and that therefore our technique has preserved only the plasmosome for the form of the one plasmosome we find, though generally spherical, quite often has the elongate chromosome shape, figured for the chromosome nucleolus (see Photo. 3, Plate I). On this point our observations support those of Moore and Robinson (05) in *Periplanata Americana*, who claim that the frequent elongation of the plasmosome is due solely to mechanical influences.

The one densely-staining nucleolar-like body which we find invariably in the first spermatocytes of our smear preparations we interpret as the homologue of the plasmosome of the egg. Photos. 1 to 4, Plate I, show it in the resting first spermatocyte, while the chromatin is still diffused

throughout the nucleus. In Photo. 3 it is elongated in form, but there can be no doubt that it is the same structure. Photos. 5 to 12, Plate I, show it still persisting during the stage when the chromatin is assuming the form of a reticulum, skein, and chromosomes. In Photo. 10, long thread-like chromosomes with a longitudinal split are clearly shown, and at the upper right hand corner of the nucleus the persisting plasmosome can be seen, though its form has been somewhat disturbed by the technique. Photos. 12 to 15 demonstrate the persistence of the plasmosome through the later stages, while the chromosomes are assuming their definite form, and Photo. 16 shows (at the right of the uppermost chromosome), the latest stage in which we find the plasmosome persisting. At these stages it is only faintly defined (as shown in Photo. 16) and when the chromosomes have attained their definite form the plasmosome has entirely disappeared—thus, in its morphology and time of disappearance it closely resembles a typical plasmosome.

As stated in our preliminary, we have not been able to find a structure in the resting spermatocyte, which we feel justified in interpreting as the homologue of the accessory nucleolus of the egg. Although we do sometimes find a small inconspicuous nucleolar-like body, in addition to the plasmosome, its presence is too inconstant to lend support to any speculation as to its possible identity with the accessory nucleolus of the egg. This small dense body is shown in Photo. 2, Plate I, below and to the right of the plasmosome and it is also differentiated in Photo. 5, below and to the right of the plasmosome. One is present also in Photos. 6 and 10, but we fear they are too inconspicuous to be brought out in the reproductions.

In all cases in which both a chromosome nucleolus and a plasmosome have been demonstrated in the first spermatocyte of *Anasa tristis*, the chromosome nucleolus has been represented as the more dense and more deeply-staining body of the two (for example, see Text Figs. 1 and 2). Morphologically, it resembles a nucleolus much more than it resembles a chromosome, and in fact, its morphological likeness to a nucleolus has been effectively used by Wilson in differentiating it from the chromosomes during the prophases, thus proving its identity with the chromosome nucleolus of the growth period. The force of this evidence is shown by a comparison of Wilson's Fig. *a* (Text Fig. 1) and Photo. 17, Plate I. The latter is a reproduction of Wilson's (05) sketch *b*, Fig. 2, reduced to a magnification of about two thousand diameters. In this section (Photo. 17) Wilson shows the plasmosome (*p*) still persisting and in addition to this the dense chromosome-nucleolus (*h*)—the structure which he interprets as a persisting, odd spermatogonial chromo-

some. A comparison of his section (Photo. 17) with our Photos. 18 to 24, Plate I, will show that all these eight preparations represent about the same stage of development. The chromosomes of his section are quite as well formed as those in our preparations—the only difference being that in his section the plasmosome still persists, whereas, in our preparations at this stage it has disappeared.

We think there can be no doubt that our Photos. 18 to 24, Plate I, represent very nearly, if not exactly, the same stage of development as Wilson's section (Photo. 17) and his sketches *c* and *d*, Fig. 2, of the same article (05). A comparison of our preparations (Photos. 18 to 24) with Wilson's section (Photo. 17) shows beyond question that there is no structure in our preparations which resembles morphologically the (odd) heterotropic chromosome (*h*) of Wilson's section. We would accentuate the fact that in our preparations *all the eleven* chromosomes are in evidence and that it is, therefore, possible to establish the identity of the chromosome which Wilson and Montgomery interpret as their odd chromosome and we are thus in a position to compare it directly with the same chromosome figured by Wilson. Unfortunately, Wilson has figured only a few of the eleven chromosomes in each of his three sketches, but this need not hamper the comparison, for all of our photographs of this stage show every chromosome, making it possible for us to identify the eccentric—and this identification is placed beyond question by the fact that the later history of this chromosome can be followed uninterruptedly.

In the very early first prophases as soon as the chromosomes attain a definite form we find as a rule nine tetrads—the two microchromosomes, (often quite far apart), and one chromosome which typically appears as two thin parallel rods. It is this last chromosome which we interpret as identical with the persisting odd spermatogonial chromosome of Wilson and Montgomery, basing this interpretation on its individuality of form which enables us to follow it uninterruptedly through the early prophase to the late prophase or metaphase, where its eccentric position outside the ring of chromosomes, establishes its identity with the odd, heterotropic chromosome—Wilson and Montgomery invariably figuring this same eccentric position for their odd chromosome at this stage.⁴ In Photo. 18, Plate I, this chromosome is in the lower left

⁴ In "The Case of *Anasa tristis*" (Science, February, 07), Professor Wilson says, that he has "for sometime had reason to suspect (in case of certain other genera) that a stage may have been overlooked in the prophases in which the odd chromosome temporarily loses its compact nucleolus-like form."

corner of the group of the eleven chromosomes, and just below the upper microchromosome. In Photo. 19 it is the curved chromosome on the left periphery of the group. In Photo. 20 it is to the right of the lower microchromosome. In Photo. 21 it is nearly in the center of the group. In Photo. 22 it is on the left periphery of the group—below and to the left of the large cross-shaped chromosome. In Photo. 23 it is just below the large cross-shaped chromosome, and in Photo. 24 it is on the left periphery of the group, just below and to the left of the upper microchromosome.

There is certainly not the remotest resemblance between this chromosome and chromosome *h* of Wilson's section (Photo. 17), nor is there a single chromosome of all the eleven which in the least resembles the chromosome *h* of Wilson's section—though there is a likeness between some of the other chromosomes of his section and those of our preparations.

Later stages in the development of the eccentric chromosome are demonstrated in the photographs of Plate II and when possible we shall compare these photographs with Wilson's and Montgomery's sketches of sections. This is, however, by no means a simple matter, for in addition to their omission of many of the stages, the chromosomes of their figures show such complete lack of detail that they resemble a group of densely-stained nucleoli, nearly all individuality of form being omitted or perhaps destroyed by their technique (Text Figs. 1 and 2).

Plate II.—In Photos. 1 to 12 the eccentric chromosome can be traced through the early prophase to the late prophase or metaphase, where it is shown in its characteristic position outside the ring of nine chromosomes. These photographs, which show the transition of the chromosomes from the early prophase (Photo. 1) to the late prophase or metaphase (Photos. 12 and 13), demonstrate that during these stages, the chromosomes may become smaller and denser, this being due evidently to contraction of the chromosomes and not to the giving off of any of their substance. In Photo. 1 the eccentric chromosome is on the upper periphery of the group and to the right of the large cross-shaped chromosome. In Photo. 2, it is just above the center of the group, being readily identified by its typical form (two thin parallel rods). In Photo. 3 it is on the lower left periphery of the group and though somewhat curved, its typical form is not obscured. In Photo. 4 it is below the group and its form in this preparation is unusual—it is a distinct tetrad, as in fact are all the ten large chromosomes, our re-

sults in this particular supporting Wilson, who also finds this chromosome showing exceptionally the tetrad form. It is difficult to conceive why this chromosome should appear even exceptionally as a tetrad, if it is destined not to divide in the second spindle, for if the tetrad form of these stages indicates a double division of the chromosome, and even if such a character appears only exceptionally it seems to us it should have the weight of positive as against negative evidence.

In Photo. 5 the eccentric chromosome is on the left periphery of the group and although its characteristic form is somewhat obscured its identity is beyond question. In Photo. 6 it is on the left periphery of the group—just above a chromosome which has separated transversely. In this preparation the typical double-rod form of the eccentric chromosome is clearly defined. In Photo. 7 it is on the right periphery of the group—just below and to the right of the upper microchromosome. In Photo. 8 it is on the left periphery of the group—just below and to the right of the upper microchromosome. In Photo. 9 it is on the upper periphery of the group—its typical double-rod form making its identification unquestionable. Just above and to the right of the eccentric is a denser chromosome showing a distinct transverse furrow, and in the following stages such a dense dyad can be identified in many of the preparations and it is clearly differentiated from the eccentric chromosome. In later stages, however (prophases of second spindle), it is possible to confuse such a dyad with the eccentric chromosome, and for this reason we would call attention to it in all these earlier stages in which such a confusion of the two is impossible. Occasionally, more than one of the ten large chromosomes has the form of a dense dyad, but the point we wish to make is that it is *not* the eccentric chromosome which assumes this form, and we shall, therefore, call attention to those preparations in which the two forms are present and clearly differentiated from each other. This is the case in Photo. 10, in which the eccentric chromosome shows its typical form of two thin parallel rods, the furrow between these rods forecasting the plane of its division in the first spindle. To the left of the eccentric is a dense chromosome with an indication of a transverse constriction and above is another chromosome contracted into almost a dyad form, yet neither of these chromosomes can possibly be confused with the eccentric. In Photo. 11 the eccentric has its position typical of the late prophase or metaphase stages, *i. e.*, outside the circle of nine chromosomes. In common with the other chromosomes at this stage, it has contracted into a smaller denser body, but the line of its

longitudinal furrow is still visible. This longitudinal furrow is shown more clearly in Photos. 12, 16, 17, 18, 19, 20, 23, etc.

Our Photos. 10 to 17 evidently represent the same stage of development shown in Wilson's sketch *k* (Text Fig. 2) and Montgomery's sketch 76 (Text Fig. 1), and a comparison of their sketches with our photographs will, we think, furnish a justification of our preference for our smear method. These authors designate this stage as the metaphase, but we have called it late prophase or metaphase because there is evidence that the chromosomes do not invariably maintain this ring position throughout the metaphase. Often cases are found in which the chromosomes are nearly or quite in a straight line at the metaphase, possible displacement of the chromosomes not offering an adequate explanation of this difference in arrangement. Photos. 20 to 27, Plate II, illustrate some of these cases. The fact, however, that the typical arrangement of the chromosomes shown for example in Photo. 12, Plate II, is often maintained at the late anaphase and telophase indicates that the ring arrangement of the chromosomes may persist through the entire division. As a demonstration of this compare Photo. 12, Plate II, with Photos. 2, 3, and 4, Plate III.

The characteristic position of the eccentric chromosome in Photos. 11, 12, 13, 16, 17, 18, 19, 20, 21, and 22, Plate II, assures its identification and makes special reference to these photographs unnecessary. In Photos. 14 and 15, however, the eccentric chromosome is not outside but takes part in forming the circle of chromosomes and we have reproduced these preparations in order to demonstrate that the eccentric, though typically, is not invariably found outside and apart from the other chromosomes. In Photo. 14 it is just below the largest chromosome of the group, its longitudinal furrow serving to identify it, and in Photo. 15 it is also just below the largest chromosome. Some of these photographs show one or two dense dyads clearly differentiated from the eccentric. In Photo. 13 we see a dense dyad in the circle of chromosomes just below the largest chromosome and almost opposite the eccentric. In Photo. 14 we again find a dense dyad in the circle and opposite the eccentric. In Photo. 15 there are two such dense dyads present, but they cannot be confused with the eccentric which is easily identified by its longitudinal furrow. A dense dyad, plainly not the eccentric chromosome, is present also in Photos. 17, 19, 22, 24, and 27. In the light of these cases we should expect to find often one or two dense chromosomes at the telophase of the first division or prophase of the second and we feel justified in interpreting such chromosomes as the daughter

chromosomes of the dense dyads of the earlier stages and when at these stages the identity of the eccentric is obscured we certainly are justified in not confounding it with these dense dyads.

The characteristic form of the eccentric chromosome with its longitudinal furrow assures its identification through the prophases, metaphase, and in some cases the anaphase of the first spindle. Beyond this stage its eccentric position is the strongest evidence of its identity, but as this relatively isolated position is typical of this chromosome in the earlier stages we may accept it as evidence of its identity in later stages also. Even in those first metaphase stages in which the chromosomes are almost in a straight line the eccentric has typically a more or less detached position and when not distinctly isolated is generally found at one end of the line of chromosomes. This is shown in Photos. 22, 23, 25, 26, and 27. In the last photograph the eccentric is at the right end of the line of chromosomes and a dense dyad is at the left end of the line, but this latter chromosome is dividing transversely and cannot be confused with the eccentric which shows its typical longitudinal furrow.

Photos. 28 to 32 are of preparations showing the early anaphase of the first division. In four of these five preparations all the eleven chromosomes can be counted and at least three of the preparations demonstrate a foreshadowing of a second division for the eccentric chromosome. We believe that the form of this chromosome during the prophases and metaphase demonstrates that it divides longitudinally at the first division while the other chromosomes divide transversely. This difference in the plane of division of the eccentric has been noted by Montgomery, 06, and figured in his sketch 138. In Photos. 28, 29, and 30 we interpret as the eccentric chromosome that chromosome which in each case is furthest to the left of the group. This interpretation we base, first, on its slightly isolated position, and second, on the fact that the plane of its division is more suggestive of a longitudinal division than is that of any of the other chromosomes. This is especially true in the case of Photo. 29, and in both Photos. 28 and 30 the two halves of this chromosome show a distinct indication of a longitudinal furrow. It is possible that this may not be demonstrated in all the reproductions, but in the original photographs it is beyond question. In Photo. 30 this same secondary furrow is demonstrated in *all* the ten large chromosomes, therefore, if our identification of the eccentric is questioned we still cannot avoid the fact that the eccentric, wherever it is, has—in common with the rest of the chromosomes—a secondary furrow, and this holds true also for Photo. 32, in which the identity of the eccentric is ob-

scured, but *all* the ten large chromosomes, without exception, show a secondary furrow. As far as we are aware, there is marked agreement among cytologists in their interpretation of the significance of the secondary furrow of the anaphase stage, and if this secondary furrow must be interpreted for some of the chromosomes as foreshadowing the plane of the second division, we certainly have a right to include the eccentric in this interpretation, and we claim for these cases in which the secondary furrow is not obscured, the weight due positive evidence as opposed to negative.

We have now reached a stage in the development of the eccentric chromosome to which we would give special attention. Previous investigators of the spermatogenesis of *Anasa tristis* have either overlooked or ignored the occurrence in the first spindle of a phenomenon which they have, however, observed in the second spindle and have interpreted as proving that one of the chromosomes of the second spindle passes undivided to one of the daughter cells. Wilson's demonstration of this phenomenon in the second spindle is shown in Text Fig. 2, *m* and *n*. Although each of these sections shows only five of the eleven chromosomes, he demonstrates between the poles of each spindle one chromosome as yet undivided. Text Fig. 1 (160 and 161) shows two of Montgomery's figures, illustrating this same phenomenon and Text Fig. 3, one of Paulmier's sections of about the same stage as Wilson's preparations *m* and *n*, shown in Text Fig. 2. A comparison of these figures with our photographs of the second spindle (34 to 42, Plate III) will demonstrate that these photographs and the sketches above mentioned represent the same phenomenon. This undivided chromosome so often seen between the poles of the second spindle we interpret as a chromosome merely retarded in division and believe that normally it finally divides. We cannot, therefore, accept the conclusion of Wilson and Montgomery that this chromosome normally passes undivided to one pole of the spindle. As mentioned above, we have been able to demonstrate that a like phenomenon may occur in the first spindle, where the fact of the final division of the lagging chromosome cannot be questioned, and we believe we must interpret the second spindle in the light of the first.

Plate III, Photos. 1 to 18 inclusive, demonstrate the presence of a lagging chromosome in the first spindle. We interpret this lagging chromosome as the eccentric chromosome of the earlier stages, and this interpretation is based on the fact of its typical position outside the circle of nine chromosomes. The force of this evidence will be appreciated by comparing Photos. 12, 13, 16, and 18, Plate II, with Photos.

2, 3, 4, 8, 10, 14 to 18, Plate III, in which the characteristic position of the eccentric chromosome is clearly shown. It seems to us that in these preparations the identification of the eccentric as the chromosome outside the circle is almost as certain as the identification of the microchromosome within the circle.

We believe that the frequent eccentric position of this chromosome offers at least a partial explanation of its retarded division. If the division of the chromosomes is dependent in any degree upon forces centered at the poles, one chromosome isolated from the group would certainly be less under the influence of this force and its response would, therefore, be slower, resulting in a retarded division. It is easy to conceive further that such a condition started in the first spindle could be exaggerated in the second, and in fact might often result in the entire omission of the division of the isolated chromosome. But we believe such a condition to be abnormal in view of the fact that we can demonstrate the division of the lagging chromosome not only in the first spindle, but also in the second. If we are right in our surmise that the more or less isolated position of this chromosome may be in a measure responsible for its frequent delay in division, we should expect to find in all forms in which dimorphism of the spermatozoa is claimed, some evidence that one member of the chromosome group may have a more or less detached position, such as we find in *Anasa*. An examination of the literature shows a very suggestive number of cases in which one of the chromosomes of the first metaphase has an eccentric position very similar to that of the eccentric chromosome of *Anasa tristis*.⁵ We shall not attempt to enumerate these cases, however, for the reason that in many of them this eccentrically-placed chromosome is not identified with the chromosome, which later, by its unequal division gives rise to the dimorphism of the spermatozoa. The force of this adverse evidence is, however, weakened when we recall that both Paulmier and Montgomery at first interpreted the lagging chromosome of the second spindle as one of the microchromosomes—its identity with the eccentric chromosome being first recognized by Wilson several years later.

Plate III, Photo. 1, shows a late anaphase or telophase of the first spindle in which a daughter half of the lagging chromosome lies midway between the poles. This chromosome belongs to the left pole, for

⁵ The possible meaning of such an arrangement of the chromosomes is of course another question, and speculation as to its significance may well be deferred until far more exact data have been collected, not only for this form, but for many more forms.

the right pole shows the complete number, eleven chromosomes, while at the left pole there are only ten, indicating that the lagging chromosome in this instance divided as we see demonstrated in Photos. 4, 8, and 9.

Although the chromosomes of these preparations show marked variation in size their identification as the chromosomes of the first division is assured by comparing them with similar stages of the second spindle, Photos. 26 to 46. In addition to the size relations the difference in the form of the chromosomes at the telophase of the two divisions is often an aid in identification, the chromosomes at the telophase of the first division, as a rule, showing a more or less complete dyad form, foreshadowing their second division. For example, the chromosomes of Photo. 10 are not much larger than those of 33, but all the chromosomes of Photo. 10 (except the microchromosomes) show the dyad form.

Photos. 2 and 3 show the eccentric chromosome only slightly retarded in its division, but in Photo. 4 the retarded condition is more pronounced. In this photograph all the eleven chromosomes are in evidence and those forming the typical circle at each pole show without exception the dyad form, the constriction which in other material is interpreted as forecasting a second division. It is most significant that in addition to these dyads one of the daughter eccentric chromosomes,—the one most remote from the pole to which it is destined,—also shows the dyad form, and we interpret this as having the same significance as the dyad form in the other chromosomes.

Photo. 5 shows two first spermatocytes at the same stage (telophase) as Photos. 2, 3, and 4. The chromosomes of the two cells have just divided forming a group of four second spermatocytes. At each of the four poles we find a lagging chromosome again unmistakably the eccentric. The point in this preparation to which we wish to draw attention is the fact that the attenuated condition of this eccentric chromosome—due perhaps partly to the technique—has accentuated the secondary furrow. We have in this preparation a somewhat exaggerated illustration of the typical secondary furrow which can be seen in the same chromosome of Photos. 4, 10, 13, 14, 15, 16, 18, 19, 20, 21, and 22—this furrow we interpret as indicating a second division for this chromosome.

Photos. 6 and 7 represent a little earlier stage than Photo. 5. The lagging chromosome of the first spindle is here shown in a position very characteristic of the lagging chromosome of the second spindle. For example, compare these photographs with Photos. 33 to 40 of the second spindle. The dyad character of the chromosomes of Photos. 6 and 7 is

obscured, due, perhaps, to an abnormal condition of the chromosomes themselves or to the technique, but we have a large number of preparations at a little later stage of this spindle, showing the same lack of detail in the eleven chromosomes at each pole. The dyad character of nearly all the chromosomes is obscured also in Photos. 8 and 9, but the chromosomes can be counted and they show the typical circular grouping with the microchromosomes within the circle and the eccentric outside. Photos. 8 and 9 show one type of the retarded division of the eccentric in which one daughter half lags midway between the poles.

In Photo. 10 the chromosomes are almost diagrammatic, both in position and form. The eccentric lies above and to the left of the largest chromosome of the eleven, one-half of this large chromosome projecting beyond the circle, but the eccentric is the only chromosome entirely outside the circle. All the eleven chromosomes except the microchromosome show the dyad form, *the secondary furrow being demonstrated in the eccentric quite as clearly as in the other chromosomes.*

Photo. 11 shows a telophase of the first spindle which illustrates a later stage of that type of retarded division of the eccentric chromosome which is foreshadowed in Photos. 4, 8, and 9, these preparations showing that in some cases one of the daughter eccentric chromosomes is destined to arrive at the pole much later than its mate. The position of the daughter eccentric in Photo. 11 bears a suggestive resemblance to the figures frequently offered in other forms as evidence that one chromosome passes over undivided to one pole. Such evidence in the second spindle of *Anasa tristis* has been given by Montgomery, 06 (Text Fig. 1, Fig. 161) and Paulmier, 99 (Fig. 36) and Wilson, 06 (Fig. 2*b*). In the telophase of Photo. 12, Plate III, the two daughter eccentric chromosomes are more equally retarded, each arriving at the pole to which it is destined at about the same time. The size alone of a lagging chromosome is not a trustworthy guide of its value, for the lagging daughter chromosome of Photo. 11 is twice as large as one of the two in Photo. 12, and yet the ten large dyads can be counted in the right pole of Photo. 11. The danger of determining the value of a chromosome by its size alone can be appreciated further by comparing, for example, the lagging chromosome of Photo. 29 with the lagging chromosome of Photo. 39—one is fully twice as large as the other, although neither has yet divided.

Photos. 13, 14, 15, 16, 17, and 18, are first telophases showing a repetition of this same phenomenon, *i. e.*, the eccentric chromosome more or less isolated from the rest of the chromosome group, and in many of these cases its dyad form is quite as pronounced as in all the

other ten large chromosomes. This secondary furrow is shown in both daughter eccentrics of Photo. 14, in one of the two of Photos. 15 and 16, and in one of Photo. 18. In all these preparations, except Photo. 17, the eleven chromosomes are clearly demonstrated at each pole.

This closes the evidence we have to offer for the presence of an eccentric and often lagging chromosome in the first spindle. We have here demonstrated it in nineteen spindles and these photographs are chosen from a larger number showing the same phenomenon. We regard the demonstration of a lagging chromosome in the first spindle as important and suggestive, especially in view of the fact that it has been overlooked or ignored by the previous investigators of the spermatogenesis of *Anasa tristis*. As stated above (p. 293), we believe that the frequently isolated position of this chromosome in the prophases offers at least a partial explanation of why it may lag in division, both in the first and in the second spindles.

Photos. 19 and 20 show a very late prophase or metaphase of the second spindle and Photos. 21 and 22 metaphases in which the chromosomes are more nearly in line. In view of the characteristic position of the eccentric chromosome in all stages in which its identity is beyond question, we feel that in these preparations where its identity is not always clear we may at least tentatively identify it as the most isolated chromosome of any given group. In Photo. 19, then, we would recognize as the eccentric, the slightly isolated chromosome at the right of the group—the eccentric of Photo. 20 as either the chromosome slightly separated from the lower left hand periphery of the group or the one slightly separated from the right periphery of the group. In Photo. 21 its identity is more obscure, but in Photo. 22 there is good reason for identifying it as the distinctly isolated chromosome at the right end of the line. The point we would make is that all the chromosomes we have identified as probably the eccentric show clearly the secondary furrow foreshadowed in this chromosome of earlier stages (pp. 288 and 291 and 294-5). Even if we are mistaken in our identification of the eccentric here we cannot escape the fact that wherever it may be in each of these groups, it shows the plane of the next division, for every chromosome, even the microchromosomes, show a constriction. The presence of the furrow might be questioned for the dense chromosome close to the microchromosome of Photos. 19, 20, and 21, because in this chromosome the constriction is so delicate it may be obscured in the reproductions. In Photo. 22, however, we believe the reproduction cannot obscure the furrow in any of the chromosomes. In any case, we do not believe that the

dense chromosome of Photos. 19, 20, and 21 should be confounded with the eccentric, for in all of these preparations its position within the group close to the microchromosome makes its identification as a lagging eccentric chromosome highly improbable, and more than this, in the earlier stages, where we find one or two dense dyads there can be no question of confounding them with the eccentric chromosome (see pp. 289-90 for a detailed description of these dyads).

Photos. 23, 24, and 25 show three anaphases of the second spindle. In these preparations the chromosomes are closely grouped, preventing the identification of each individual chromosome, but in all these preparations, the evidence points to a division of *all* the chromosomes.

Photo. 26 shows a later anaphase of the second spindle. The eccentric chromosome can be identified on the left periphery of the groups by its typical position outside the two circles of chromosomes. It has not yet divided, but its dyad form and distinct transverse furrow, we believe, can have no other meaning than forecasting a division. This evidence is repeated in Photo. 27. In this preparation the eccentric has the same typical position shown in Photo. 26, and its dyad form is plainly demonstrated.

Photo. 28 shows the eccentric chromosome *after* its division has occurred. Its typical position, outside the two circles of chromosomes assures its identification, and we must interpret it as the eccentric chromosome, the division of which is so clearly foreshadowed in Photos. 26 and 27. Only nine of the ten large chromosomes are clearly differentiated at each pole of this spindle, but it is quite superfluous to even comment upon the possibilities of individual chromosomes being obscured in such small spindles and especially in view of the fact of the tendency of the chromosomes of the second telophase to contract at once into almost a solid mass of chromatin (see Photos. 30, 31, and 46). It is only when the eccentric is retarded in division, that the contraction of the chromosomes at the poles is often delayed and the individual chromosomes are differentiated. It is instructive to compare the three Photos. (26, 27, and 28) with telophases of the *first* spindle, for example, Photos. 2, 3, 10, and 14 to 18.

In Photos. 29, 30, and 31 we have another series of photographs showing the division of the eccentric chromosome of the second spindle. In Photo. 29 every chromosome is shown, and the dyad form of the lagging (eccentric) chromosome is so pronounced that it is almost a demonstration of its division. In Photo. 30 the division of the lagging chromo-

some has actually occurred, and in Photo. 31 the complete separation of the daughter halves is demonstrated.

Photos. 32 to 39 are telophases of eight second spindles, all of them showing the eccentric chromosome ready to divide though its division is retarded. The opposite poles of each spindle are still nearly in contact and in Photos. 35, 36, 38, and 39 the dyad form of the retarded chromosome foreshadows its ultimate division. It is instructive to compare these preparations with Photos. 6, 7, and 5 of the first spindle.

In Photos. 32, 33, and 34 we see the lagging chromosome gradually assuming the position necessary to its transverse division and in Photo. 35 this position is reached, and we may well question the significance of this turning, if this chromosome were destined not to divide at all. This turning of the lagging chromosome into a position necessary for its transverse division is another evidence of its identity with the eccentric of earlier stages, for we have demonstrated that in the first spindle this chromosome divides longitudinally while all the other chromosomes divide transversely, and we would, therefore, expect in the second spindle to find this chromosome dividing transversely while the others divide longitudinally. The fact that this chromosome divides transversely while all the others divide longitudinally may be another factor in causing its frequent retarded division.

In Photo. 35 the eccentric is in position to divide almost as soon as the other chromosomes, and we believe that normally the division of the eccentric takes place before the poles are so far apart as we see them, for example, in Photos. 42 to 44. Although the two poles of the spindle are much further apart in Photos. 40, 41, 42, and 43, even in these photographs we have evidence that in such preparations the division of the eccentric, though greatly delayed, may yet occur. There are two facts pointing to this, the dyad form of the eccentric chromosome and the presence of a fiber attaching it to *both* poles. In Photo. 40 both fibers are seen—each end of the lagging chromosome being connected by a fiber to opposite poles of the spindle—the pointed ends of the eccentric chromosome to which the fibers are attached denoting that the two halves are being pulled in opposite directions. This feature is shown again in Photo. 41, though the fibers are so faint they will probably not be brought out in the reproductions. In Photo. 42 both fibers are in evidence and the one is most distinct which connects the eccentric with the pole from which it is most remote. This photograph shows, in addition to the fibers, one end of the eccentric chromosome pulled to a sharp point and also the secondary furrow forecasting its division. In Photo.

43, the fibers are obscured, but the eccentric shows an indication of the dyad form. The presence of fibers connecting the ends of the lagging chromosome to opposite poles of the second spindle has been represented by Paulmier and one of his figures showing this feature we have reproduced in our Text Fig. 3. His confounding this chromosome with one of the microchromosomes does not weaken the significance of the fact that this chromosome is connected with a fiber to *both* poles of the spindle. In describing the effect of these fibers on the form of this chromosome he says: "It is somewhat elongated as if stretched by the pull of the opposite spindle fibers which are attached to it," p. 244.

The telophase of the second spindle shown in Photo. 44 demonstrates that the eccentric chromosome may divide even when the poles of the spindle are very far apart for the daughter halves of the lagging chromosome have almost completely separated. In this preparation the daughter cells are still attached though the constriction of the cytoplasm which precedes cell division has appeared between them. Probably this point may not be brought out in the reproductions.

In Photos. 45 and 46 the final division of the lagging (eccentric) chromosome is again shown. In Photo. 46 the identity of the lagging chromosome is more obscure, but the fact that one of the chromosomes has not yet contracted as much as the others, added to its eccentric position, point to its identity as the lagging chromosome. This photograph shows also that the poles of the spindle have turned after its division—a feature common for both the first and second spindles. Photo. 51 shows two resting spermatids, each with a nucleolus.

We believe that these twenty-one preparations of anaphases and telophases of the second maturation division demonstrate that it is not safe to assume that because a chromosome is retarded in division it necessarily follows that it will not divide at all. It is a fact that the main part of the evidence that has been offered to establish the theory of the dimorphism of the spermatozoa is a demonstration that at the late anaphase or telophase of a first or second maturation division one chromosome is often found, either exactly between the poles or nearer one pole than the other, and in nearly, if not all, of these cases, only a few of the total number of chromosomes are shown.

Examples of this kind of evidence for *Anasa tristis* are reproduced in Text Figs. 1, 2, and 3. Montgomery's sketch 161 (Text Fig. 1) together with the metaphase of his sketch 160 are the only figures he has given us in evidence of the dimorphism of the spermatozoa of *Anasa tristis*. Paulmier's Fig. 35 (Text Fig. 3) is another example of this

kind of evidence, and in addition to this figure he has given us also three or four sketches showing second telophases with the chromosomes at each pole massed beyond individual recognition, and at one pole of each figure he shows a chromosome somewhat isolated from its fellows. Such an inextricable massing of the chromosomes at the poles is even more pronounced in Montgomery's sketch 161 (Text Fig. 1) and it seems to us very hazardous to draw such important conclusions from these stages unless all the chromosomes are in evidence. For example, our Photo. 11, Plate III, might be offered as evidence that in *Anasa tristis* it is the unequal distribution of chromatin in the *first* division which establishes the dimorphism of the spermatozoa, but in the light of Photo. 12 such an interpretation becomes untenable, even if we did not have the additional evidence of the large number of photographs where the division of *all* the chromosomes of the first spindle is absolutely demonstrated. Even in Photo. 11 ten large dyads can be counted in the right hand pole of this telophase, only the microchromosomes being completely obscured and, as stated above, p. 295, the size of the daughter eccentric of Photo. 11 is not a safe guide in determining its value. *M* and *n* of Text Fig. 2 are reproductions of Wilson's sketches of the second spindle. In each of these figures he shows five of the eleven chromosomes. In his later paper (06) he has added to the evidence on this point his sketch *b* of Fig. 2 showing the eccentric at one pole, *i. e.*, with six of the eleven chromosomes at one pole and five of the eleven at the opposite pole, and besides this two more sketches (*c* and *d*) which he interprets as sister groups of a second spindle—one pole (*c*) showing ten chromosomes and the other pole (*d*) eleven. Wilson's sketches *m* and *n* (Text Fig. 2), Montgomery's sketch 161 (Text Fig. 1), and Paulmier's sketch 35 (Text Fig. 3) bear a sufficient resemblance to some of our photographs to admit of comparison. They undoubtedly represent the same phenomenon as shown in our Photos. 32 to 42 and we believe these photographs should be interpreted in the light of those in which we have been able to demonstrate the division of this lagging chromosome, *i. e.*, Photos. 26 to 31 and 44, and in the light of all the additional data we have been able to offer in support of our belief that normally the eccentric chromosome divides in both spindles.⁶

In the above detailed description of the eccentric chromosome we have followed its development from the earliest prophase of the first sperma-

⁶ In at least two forms, outside the Hemiptera, a structure identified as an accessory chromosome has been shown to divide in *both* spindles and further, is figured as a lagging chromosome.

toocyte in which its identity is assured, through the prophases, metaphase, anaphase, and telophase of *both* divisions. We believe we have shown good reason to doubt the interpretation of Wilson and Montgomery that normally this chromosome does not divide in the second spindle, but passes over undivided to one of the daughter cells. The evidence that our preparations furnish for this position is given in full in the above detailed description of the eccentric chromosome and we shall close this description with a brief summary of the important points:

First, in the resting first spermatocyte we see no evidence of a persisting spermatogonial chromosome—we find *all* the chromosomes without exception following the typical course of development which has been demonstrated for so many forms, *i e.*, through this period the identity of the individual chromosomes is completely obscured—the chromatin being diffused throughout the nucleus, later forming a chromatin reticulum which passes through definite changes culminating in the reappearance of individual chromosomes.

Second, we find *only one* body in the rest stage that in any way resembles the dense deeply-staining nucleolar-like structure interpreted by Wilson and Montgomery as a persisting spermatogonial chromosome and this body we interpret as a plasmosome and not a chromosome.

Third, in the prophases of the first spermatocyte we have been able to demonstrate a special chromosome (which we have called the eccentric chromosome) persistently maintaining a form which assures its identification throughout these stages, and this chromosome does not in the least resemble the body figured by Wilson as the heterotropic (eccentric) chromosome of these stages.

Fourth, although the eccentric chromosome of the prophases usually appears as two thin parallel rods we have been able to demonstrate that it may also appear as a tetrad, showing the secondary furrow common for the other large chromosomes—the furrow interpreted in all other forms as foreshadowing the plane of the second division.⁷

Fifth, we have demonstrated the secondary furrow in the eccentric chromosome not only in the prophase of the first spindle but at the anaphase and telophase of this spindle and at the prophase and metaphase of the second spindle, this secondary furrow or constriction

⁷ Wilson has observed the exceptional appearance of the tetrad form of this chromosome, and Paulmier mentions its tetrad form without any note of its being exceptional.

giving this chromosome the dyad form which is typical of the other chromosomes at this stage.

Sixth, the interpretation that this secondary furrow foreshadows a second division is confirmed by the demonstration of the actual division of the eccentric chromosome—this proving that the delay in the division of this chromosome does not necessarily mean the omission of its division.

Seventh, we have demonstrated that a delay in the division of the eccentric chromosome may occur also in the first spindle and we have shown that in certain phases characteristic of the behavior of the lag-

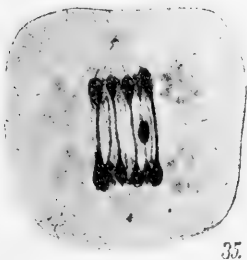


FIG. 3.

TEXT FIG. 3. Reproduction of one of Paulmier's sketches of the second spindle of *Anasa tristis*, 99.

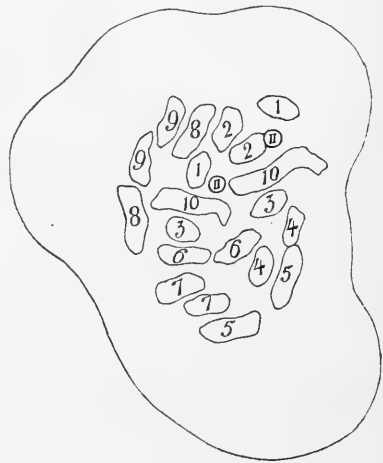


FIG. 4.

TEXT FIG. 4. Diagrammatic reproduction of Photo. 47, Plate III. Enlarged one diameter.

ging chromosome in the second spindle we have a striking repetition of what may occur in the first spindle and we have given reason for believing that the typical eccentric position of this chromosome in the early stages as well as the difference in its plane of division may be in a measure responsible for its frequent retarded division in both the first and second spindles.

The Spermatogonial Chromosomes.—If we are right in our belief that the eccentric chromosome of the first spermatocyte has the same value as the other chromosomes—that it is a bivalent and not a univalent as interpreted by Wilson and Montgomery—that it represents

two spermatogonial chromosomes rather than one, then we should be able to add to the evidence given above for the second division of this chromosome, the further evidence of a demonstration of twenty-two chromosomes in the spermatogonia instead of twenty-one as figured by Wilson and Montgomery. This evidence we give in Photos. 47 to 50, each preparation showing twenty-two spermatogonial chromosomes.

Our appreciation of the difficulty of demonstrating the number of chromosomes in the spermatogonial cells we expressed in our preliminary note as follows: "We realize in common with all cytologists the difficulty of getting a correct count of so large a number of small bodies crowded into a contracted space. If two or more chromosomes are in such close contact that their line of separation is obscured a correct count is impossible. It is certainly possible to find cells in which only twenty-one chromosomes can be differentiated and still easier to find cells in which only twenty or nineteen are defined. It is much more difficult to find each chromosome so distinctly isolated that all can be demonstrated in one photograph." We have, in fact, many photographs of *Anasa* in which only eighteen, nineteen, twenty, and twenty-one spermatogonial chromosomes can be counted, but we have not thought it necessary to publish these for such negative evidence merely illustrates one of the elementary difficulties encountered by every cytologist.

We believe the early spermatogonial prophase the most favorable period for an accurate counting of chromosomes, for at this stage the chromosomes are less closely grouped and have not yet begun to contract into the smaller denser forms they assume just before division. This is shown in the first spermatocyte by comparing Photos. 21 to 24, Plate I, with Photos. 13 and 14, Plate II, and the extent of such contraction of the spermatogonial chromosomes is illustrated by comparing Photo. 47 with Photo. 50. The prophase shown in Photo. 47 clearly demonstrates twenty large chromosomes and the two microchromosomes one of these attached to one of the large chromosomes. The position of these microchromosomes is shown in a diagrammatic sketch of this photograph enlarged one diameter, reproduced in Text Fig. 4. An enlarged photograph was taken of the original negative and Text Fig. 4 was traced from a print of this enlarged negative. The individual chromosomes are numerically paired in this sketch merely to emphasize the absence of an odd, unpaired chromosome—but we have made no pretense to accurate pairing of the many chromosomes which show only a slight variation in size. Twenty-two spermatogonial chromosomes are again demonstrated in Photos. 48, 49, and 50. Just below the upper

left periphery of the group of chromosomes of Photo. 50 there are two chromosomes in contact, they are nearly at right angle to each other, and although in close contact their individuality is by no means obscured. Both in the preparation and the original negative it is impossible to interpret these as a single chromosome and we hope their differentiation will not be obscured in the reproductions. We have selected for reproduction this example of a group of spermatogonial chromosomes, showing two of the chromosomes in contact, because we want to point out that with an unfavorable technique two such chromosomes would inevitably be fused into one.

How much detail can be lost by the shrinkage of fixation may be appreciated by comparing, for example, the metaphases of Montgomery's sketch 76 (Text Fig. 1) and Wilson's sketch *k* (Text Fig. 2) with our photographs of the same stage on Plate II. Such a loss of detail would inevitably obliterate the differentiation of two chromosomes in such close contact as the two shown in Photo. 50.

We should like to point out here that we have found the demonstration of twenty-two oögonial chromosomes in *Allolobophora* quite as difficult as the demonstration of twenty-two spermatogonial chromosomes in *Anasa*. The size of the cells under investigation and the technique are certainly important factors in estimating the number of chromosomes. With pricked eggs of *Allolobophora* the eleven bivalents of the first oöcyte can be demonstrated without any difficulty,⁸ but even with this method the full number of chromosomes in the extremely small oögonial cells may often elude patient search.

We have, perhaps, a further demonstration of these difficulties in *Lumbricus terrestris*. In Calkin's, 95, work on the spermatogenesis of *Lumbricus terrestris* he gives the number of first spermatocyte bivalents as sixteen and his count has been corroborated by Bugnion and Popoff (05). We have not studied the spermatogenesis of *Lumbricus terrestris* but in the pricked eggs of this form we have demonstrated many more than sixteen perfect tetrads and we hope later to publish a few photographs of these preparations.

The reality of the obstacles met by the cytologist in an effort to count accurately the number of chromosomes can be appreciated by recalling how often investigators working on the same material have arrived at different conclusions, disagreeing in their estimate sometimes to the point of doubling the number. It is impossible to appreciate these diffi-

⁸ Foot and Strobell, 05, photos. 116 to 130.

culties and hold unshaken faith in a theory that must fall with the miscout of a single chromosome.

Individuality of the Chromosomes.—The first step in discussing this point is to give a definition of one's conception of the term Individuality, for this term has been very loosely employed. If we mean by "Individuality of the Chromosomes" merely that we recognize certain characteristics of size and form in some of the chromatin units called chromosomes and that there is a frequent repetition of these forms during different stages of development, then we may claim that the chromosomes of *Anasa tristis* unqualifiedly support the theory of the "Individuality of the Chromosomes." But on the other hand, if by "Individuality of the Chromosomes" we claim their morphological continuity, that several or even only one of the chromosomes can be followed *uninterruptedly* from the spermatogonium to the spermatid, that even during the growth period the chromosome form is maintained, then we must say that in our preparations *Anasa tristis* supports in a very restricted sense, if at all, the theory of the "Individuality of the Chromosomes."

If at any time during the growth period it can be demonstrated not only that the chromosome form is obscured but that in its place we find a very different, though equally definite, chromatic structure—chromatin granules, a chromatin reticulum or a chromatic skein, we have no right to assert that the chromosomes of the earlier and later stages have retained their individuality intact during this period.

Although in *Anasa tristis* it can be demonstrated that during the rest stage of the first spermatocyte the morphological identity of the chromosomes is completely obscured, it can be demonstrated with equal clearness that among the eleven chromosomes which emerge from the resting first spermatocyte three forms are so conspicuous and are so often repeated that as a rule they can be identified from the early first prophase to the telophase of the second spindle. These are the eccentric chromosome, the microchromosomes, and the largest chromosome of the eleven which is typically cross-shaped. To these might be added the dense dyad described on p. 289, but this form is neither so distinctive nor so constant as the other three forms.

The eccentric chromosome we have already traced from the very early prophase to the telophase of the second spindle.

The microchromosomes are so conspicuous in nearly all the photographs of the three plates that it seems unnecessary to trace them through the individual photographs. We will draw attention later to certain exceptions in their form or size.

The large cross-shaped chromosome can be identified in nearly all the photographs, though in many its characteristic shape is more or less obscured. In Plate I, Photos. 18 and 21 to 24, the cross form is clearly defined, also in Photos. 1, 3, 4, 5, 6, and 8 to 12, Plate II. In many of the later stages on this plate the cross form is not so clear but it is well defined in Photos. 20 to 26 and in all the cases in which the cross form is obscured this chromosome can be identified by its size. At the telophase of the first spindle and prophase and metaphase of the second spindle it can be identified as the largest dyad of each group, its size making its identification often possible even as late as the telophase of the second spindle. In a recent work on the prophases of the egg of *Allobobophora* we showed that cross-shaped chromosomes could be formed by the contraction of two rod-shaped univalents. We described the process as follows: "They undoubtedly arise by a simple contraction of a bivalent chromosome, *i. e.*, two rod-shaped univalent chromosomes placed end to end. As they contract and are pressed together each splits open along the line of the longitudinal furrow; the ends are thus pressed out at right angles forming the two arms of the cross."³ The shape of the largest chromosome in the group of Photo. 2, Plate II, indicates that the cross-shaped chromosome in *Anasa* may be formed in the same way and we also find transitional stages between the two longitudinally split rods and completely formed crosses. In *Anasa* these transitional stages are very common for all the nine chromosomes which often have this cross form, and these stages are shown in the early and late prophases and metaphases of Plates I and II. There are very few of these preparations, which show exactly the same number of well-defined crosses or split rods, and the transitional stages between the two forms are too obvious to need detailed description. We would accentuate the fact of the occurrence of such transitional forms because the omission of such forms in other material has been especially noted.

The large chromosome of *Anasa* exceptionally shows a form quite different from the cross, but we believe that in each case its relation to the cross form is evident. Such exceptions are seen in Photos. 19 and 20, Plate I. These two chromosomes, each the largest of the group, appear as two longitudinally split rods placed *side by side* instead of end to end, and thus they seem to foreshadow two longitudinal divisions. We believe, however, that they can be interpreted in the light of the large cross-shaped chromosome of Photo. 18. A further step in closing the

³ Foot and Strobell, 05, p. 219.

space between the arms could produce the forms of Photos. 19 and 20. A third exception to the well-defined cross form is seen in the largest chromosome of Photo. 7, Plate II, but there is no evidence that this does not represent merely a condensation of the two halves of a cross-shaped chromosome.

Quite as marked a variation in form as well as size may be demonstrated also for the microchromosomes. The extent of such variation can be appreciated by comparing the microchromosomes of the early prophase of Photos. 2 and 3, Plate II. Those of Photo. 3 besides being different in form appear to be fully twice as large as those of Photo. 2. We might expect this if all the other chromosomes were proportionately larger, but in fact they are, if anything, smaller than those of Photo. 2. Such individual and independent variation in size seems to challenge us to show a like variation in both the small spermatogonial chromosomes, or it suggests that although the chromatin may emerge from the resting spermatocyte showing the typical number of subdivisions, the size relations of these chromosome units may be only approximately maintained.

The thread-like microchromosomes of Photo. 3, Plate II, seem to offer an explanation of Montgomery's observations as to the difference in size of these two chromosomes. A section showing a transverse view of one and a longitudinal view of the other would demonstrate the two showing a marked inequality in size. Even a smear may show a slight inequality in the size of these chromosomes, but in such cases one of them is obviously more condensed than the other. This is shown in Photo. 4, Plate II, but a comparison of the large number of photographs in which the two are clearly defined proves that in our preparations the two univalent halves of this bivalent are equal in size.

In considering the individuality of the chromosomes, it is an interesting fact that the one chromosome which divides longitudinally in the first division, divides transversely in the second, and it is a point in support of the theory that a longitudinal and transverse separation of each chromosome may have an important significance.

The history of the eccentric chromosome indicates that we may rely on the evidence of the prophase in determining the plane of the next division, for example, the prophases of the first spermatocyte of Photos. 21 to 24, Plate I, clearly predict that in the first spindle the eccentric will divide longitudinally and the other chromosomes transversely and this proves to be true. The fact that this can be demonstrated gives us good reason to question certain apparent contradictions, *e. g.*, the form of the chromosomes of Photos. 19 to 22, Plate III, would indicate that those

chromosomes which divided transversely in the first division are to divide transversely also in the second division, but in the light of the early first prophases, it seems more reasonable to interpret the elongated form of the chromosomes in Photos. 19 to 22 as due to other causes, rather than to believe that the line of division so clearly marked out in the early stages should be abandoned and the chromosomes divide at right angles to it. This would hold true also for those exceptional cases in which the eccentric chromosome appears to divide transversely in the first spindle.

Any investigation of the individuality of the chromosomes involves a consideration of the theory of the conjugation of the paternal and maternal chromosomes and no study of the chromosomes of a special form can be even approximately thorough, without an attempt to point out the relation in which it stands to this popular theory. As in the case of the more general theory of the individuality of the chromosomes it is first important to define the kind of evidence one considers necessary to support the theory in question. If one is satisfied with the evidence that because so many of the spermatogonial chromosomes are duplicates, we may safely conclude that all are duplicates and that each bivalent of the first spermatocyte is composed of two duplicate univalents, many of these resembling the spermatogonial duplicates, then we may feel justified in saying that *Anasa* unqualifiedly supports the theory of the conjugation of paternal and maternal chromosomes. But we do not feel that such evidence justifies us in claiming that *Anasa* stands for this theory unless we can support it by the further evidence of a complete continuity of the chromosomes between the stages of paired spermatogonial chromosomes and spermatocyte bivalents. On the contrary, the evidence points rather to a complete disintegration of every chromosome unit during this period of the resting spermatocyte and we have, therefore, no right to affirm that there is no commingling of the chromatin during this stage—that the individuality of each chromosome is maintained.

It seems to us that some reliance can be placed on the preparations shown in Plate I, Photos. 1 to 12, where it is clearly demonstrated that the individual chromosomes have lost completely their chromosome form and in their place we find chromatin granules, a chromatin reticulum, or a chromatic skein. It is certainly not unreasonable to believe that at this period the substance of the chromosomes may become commingled and transformed into the above-mentioned chromatic structures, plainly visible under the microscope, and that it finally emerges from this period

of transformation as eleven bivalents, each approximately equal in size to a pair of spermatogonial chromosomes. The duplicate chromosomes of the oögonial and spermatogonial cells may offer a demonstration of the unquestioned fact that the chromatin is contributed equally by both parents, but it seems to us this by no means involves a demonstration of the theory that the bivalents of the first spermatocyte are formed by a *conjugation* of these original oögonial or spermatogonial pairs. Again, although the univalent halves of the bivalents of the first spermatocyte certainly forecast a later equal division of the chromatin of each bivalent, we do not believe that this necessarily includes a further demonstration that each bivalent is in fact the two spermatogonial chromosomes with which it may approximately agree in size,—that these chromosomes have remained intact during the period in which the chromatin of all the chromosomes is apparently commingled. Have we a right to affirm that there is no readjustment of the chromatin during this period?

The unusual form of the two microchromosomes of Photo. 3, Plate II, has a very significant bearing on the question of how much meaning we may attach to the duplicate halves of each bivalent. It is evident that these two microchromosomes not only differ in form from those demonstrated in any of the other preparations of the same stage, but they are fully twice as large. As mentioned above, we might expect this increase in size if all the other chromosomes were proportionately larger, but they are in fact rather smaller than those of other preparations of this stage. Further, the unusual size and form of the microchromosomes of Photo. 3, Plate II, cannot be adequately explained on the ground of faulty technique for that would involve distortion and increase in size for all the other chromosomes of the same group and there is no evidence of such disturbance.

If we must interpret these two unusual microchromosomes in accordance with the theory of the conjugation of paternal and maternal chromosomes, we are forced to the conclusion that not only two individuals—male and female—can show *exactly* the same marked variation in the *same* chromosome, but that two such rare cases should unite. We avoid these difficulties, however, if we assume that the apparent disintegration of the spermatogonial chromosomes during the rest stage is a reality and that the chromatin emerges from the rest stage with each bivalent only approximately the same size as a pair of spermatogonial chromosomes.

A comparison of our photographs—all taken at the same magnification—will show some ground for this assumption, that individual bivalents resemble only approximately a pair of spermatogonial chromosomes.

An actual identification of the spermatocyte bivalents with the spermatogonial pairs would demand that the *relative* size of the eleven bivalents of a group should be as constant as the *equality* in size of two daughter bivalents and this we do not find. If we would avoid this difficulty by assuming paternal and maternal individual differences, then we should seldom find two daughter bivalents exactly like, but this equality of daughter bivalents is a conspicuous fact.¹⁰

An inconsistency in the relative size of the largest cross-shaped bivalent is seen by comparing Photos. 5 and 6, Plate II. In Photo. 6, there is not a marked difference in the apparent amount of chromatin in the large cross-shaped chromosome, and the one (next in size) just below and to the right of it, but in Photo. 5, on the contrary, there is much more contrast in size between the largest chromosome and the eight tetrads surrounding it. Greater variations in the relative size of chromosomes are common, though we find no such inconsistency in the daughter halves of bivalents. We would interpret the halves of each bivalent as identical with the daughter chromosomes of the first division rather than with definite individual spermatogonial chromosomes. It seems to us that the precocious separation of daughter halves—as shown in the microchromosomes—does not necessitate interpreting them in accord with the theory of the conjugation of paternal and maternal chromosomes.¹¹ It is certainly very significant that we do not find unequal daughter bivalents, each daughter half invariably indicating as equal a division of chromatin as is represented in the daughter chromosomes of any anaphase or telophase—and yet we constantly find a variation in the relative size of entire bivalents, such variations making size relations of a chromosome a most uncertain guide for identification, unless the difference in size is so extreme it allows for individual variation, as for example, the microchromosomes. Compare the lagging chromosomes of Photos. 29 and 39, Plate III, as an illustration of the variation in size that may be shown by the same chromosome at the same stage of development. We have pointed out these two photographs for comparison because the chromosomes at the poles of the two cells do not show any such marked contrast in size as is demonstrated in the eccentric chromosomes. The difference in size of these two lagging

¹⁰ If we assume that this frequent variation in the relative size of bivalents is due to the technique then it should produce a like effect on daughter bivalents, and this we do not find.

¹¹ In some cases the microchromosomes of the early prophases are nearly in contact. See Photos. 13 and 23, Plate I.

chromosomes can scarcely be wholly accounted for on the ground of contraction alone. The eccentric chromosome of Photo. 39 also shows a relative inequality in size—the inconsistency of its size in relation to the other chromosomes is even greater than that mentioned above for the large cross-shaped bivalent of Photos. 5 and 6, Plate II. These remarkable inconsistencies can be fully appreciated only by more exhaustive work on each form, and we have not yet been able to undertake this in *Anasa*. In our selection of cells to illustrate the phenomenon of the lagging chromosome we have made no special effort to demonstrate individual variations, but we hope to continue our study of *Anasa tristis* with the view of giving more exact data on this point. Individual variations, however, can scarcely be called upon to explain away the evidence we have given to demonstrate the division of the lagging chromosome of the second spindle.

These variations, which cannot be wholly explained by technique or contraction, offer a tempting invitation to premature speculations. The variations we find not only in the size of individual groups of the same stage but between members of the same group, and also variation in the individual form and in the grouping of the chromosomes seem to offer quite as plausible a field for speculation as to the relation of these variations to the adult individual, as much of the evidence we have been asked to consider as a morphological explanation of other vital phenomena.

We do not underrate the fact that the phenomena of Idiochromosomes observed by Miss Stevens, 05, and Wilson, 05, present a strong case in favor of the theory of the conjugation of paternal and maternal chromosomes, but in this paper our discussion of this theory is clearly limited to an attempt to point out its bearing on the phenomena as we find them in *Anasa tristis*.

NEW YORK, May, 1907.

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EXPLANATION OF PLATES.

All the photographs, except No. 17, Plate I, were taken from our modified smear preparations of the testes of *Anasa tristis* at a magnification of 1000 diameters. The Zeiss Apo., 2 mm. immers lens, 140 apr. and compensating ocular 4 were used with camera draw of $25\frac{3}{4}$ inches.

The preparation were stained with a saturate solution of Bismark brown.

The reproductions are bromide prints made by the Rotograph Company from our own negatives.

PLATE I.

PHOTO. 1. Rest stage of spermatocyte 1st order, showing diffused chromatin and round plasmosome.

PHOTO. 2. A little later stage than Photo. 1. The chromatin in this cell shows a slight indication of a network and the plasmosome has the typical round form.

PHOTO. 3. Resting spermatocyte about the same stage as above showing an elongated plasmosome.

PHOTOS. 4 to 8. Resting spermatocytes showing different stages in the formation of the chromatin network. All the plasmosomes have the typical round form.

PHOTO. 9. In this spermatocyte the heavy chromatin reticulum shows a longitudinal split in several places. The persisting plasmosome is somewhat distorted by the technique.

PHOTO. 10. A little later stage, where the chromatin is in the form of loops, many of them showing a longitudinal split. The plasmosome at the upper right hand periphery has been somewhat distorted by the technique.

PHOTOS. 11 AND 12. At this stage the chromosomes are beginning to assume a definite form, and the round densely staining plasmosome is still present.

PHOTOS. 13, 14, AND 15. Later stages where the form of the chromosomes is becoming clearer, and some of the characteristic shapes can be recognized. In Photo 13 an abnormal condensation of the chromatin can be seen in several chromosomes. The persisting plasmosome is present, on the upper periphery of the three preparations.

PHOTO. 16. A little later stage where the form of the chromosomes is more compact. Close to the chromosome on the upper periphery a faintly stained plasmosome is seen. This is the latest stage at which we have demonstrated the plasmosome.

PHOTO. 17. \times about 2000. Reproduction of Wilson's drawing *b* from his Figure 2 in "Studies on Chromosomes," II. Section of "spermatocyte nucleus of *Anasa tristis* late growth period," showing 5 chromosomes and structure marked *h*, the odd, heterotropic chromosome. Persisting plasmosome indicated under *p*.

PHOTOS. 18, 19, AND 20. Early prophases about the same stage shown in Wilson's figure (Photo. 17). In these preparations all 11 chromosomes are present. For identification of eccentric chromosome in these photographs, see page 287.

PHOTOS. 21 TO 24. Early prophases. All the 11 chromosomes are present and each preparation shows 9 tetrads, the 2 microchromosomes, and the eccentric chromosome, which can be readily recognized by its form,—two parallel rods straight or sometimes slightly curved. For detailed description of this chromosome, see page 287.

PLATE II.

PHOTOS. 1, 2, AND 3. Early prophase showing characteristic shapes of the chromosomes. The eccentric chromosome can be recognized by its typical form of two parallel rods. In Photo. 3 the microchromosomes are unusual in size and form.

PHOTO. 4. Early prophase. In this preparation *all* the ten large chromosomes, including the "eccentric" are tetrads.

PHOTOS. 5 TO 10. Later prophases, when the chromosomes condense and are somewhat smaller than at the early prophase. In all these preparations the eccentric chromosome can be identified by its characteristic form.

PHOTOS. 11 TO 19. Late prophase or metaphase showing the typical ring arrangement of the chromosomes with the eccentric as a rule, outside the circle. The eccentric retains its typical form in all these preparations, although at this stage the rods become shorter and thicker.

PHOTOS. 20 TO 27. Illustrating one type of preparation for the first spermatocyte division. The photographs show successive stages of the ring opening to form the metaphase. Through all the steps of this process, the eccentric chromosome maintains a position more or less detached from the other chromosomes. It can be recognized on the extreme left of Photos. 20 to 23, and on the extreme right of Photos. 25, 26, and 27.

PHOTOS. 28 TO 32. Early anaphases of the first division. In 28, 29, and 30 the eccentric is on the left of each group of chromosomes. In Photos. 30 and 32, all the ten large chromosomes, including the eccentric show a transverse as well as a longitudinal furrow.

PLATE III.

PHOTO. 1. Late anaphase or telophase of first division. Eleven chromosomes are shown at the pole on the right while only 10 have reached the opposite pole, the eleventh (half of the divided eccentric chromosome), lagging midway between the poles.

PHOTOS. 2 AND 3. Telophase of first division, showing both poles of the spindle. The position of the daughter eccentric chromosomes on the left of each group, demonstrates their slightly retarded division.

PHOTO. 4. Telophase of first division. Ten chromosomes are clearly shown at each pole, with the divided eccentric lagging between the poles, one of the daughter eccentrics showing a distinct transverse furrow.

PHOTO. 5. Two telophases of the first division. In both preparations the eccentric chromosome has lagged in division, and at each pole the daughter eccentric shows an exaggerated transverse furrow. See p. 294 for further description.

PHOTOS. 6 AND 7. Telophase of first division showing the undivided eccentric chromosome lagging between the poles. (See photo. 5 for a little later stage.) Compare these with Photos. 33, 34, 36, 37, 38, and 39 for similar phenomenon in second division.

PHOTOS. 8 AND 9. Telophase of first division. In each preparation one of the daughter eccentric chromosomes is lagging midway between the poles.

PHOTO. 10. Telophase of first division. Eleven chromosomes shown at each pole, all, even the lagging daughter eccentric chromosomes, showing a transverse furrow. Our preparations show at both poles of these first spindles the same remarkable uniformity of the grouping of the chromosomes characteristic of the late first prophase or metaphase. See Photos. 11, 12, 13, and 16, Plate II. And we demonstrate this same arrangement of the chromosomes at the telophase of the second spindle (see Photos. 33, 35, 36, 38, and 43, Plate III).

PHOTO. 11. Later stage of the type of retarded division of the eccentric chromosome foreshadowed in Photos. 4, 8, and 9, which results in only one of the daughter eccentrics being retarded in reaching the pole—the whole figure bearing a suggestive resemblance to the demonstration offered in other forms, that one chromosome passes over undivided to one pole.

PHOTO. 12. Same stage as Photo. 11. In this preparation both the daughter eccentric chromosomes are equally retarded in reaching the poles.

PHOTOS. 13 TO 18. Telophases of first division, showing 10 chromosomes at each pole, and the daughter eccentric chromosome in its characteristic position outside the ring, and in nearly all the preparations this chromosome shows the transverse furrow. In all the groups, the interesting duplication of the form and arrangement of the chromosomes at both poles is clearly brought out.

PHOTOS. 19 AND 20. Prophases of second spindle. All the chromosomes in these preparations have the dyad form, typical of this stage. Compare with the tetrads of the first spindle prophase.

PHOTOS. 21 AND 22. Metaphases of second spindle. The isolated chromosome on the extreme right of Photo. 22, which we identify as probably the eccentric, shows a clear transverse furrow, and a like furrow or constriction is demonstrated in *all* the chromosomes of both these preparations.

PHOTOS. 23, 24, AND 25. Three anaphases of second spindle. Although the chromosomes in these preparations are closely grouped, the division of all the chromosomes is plainly indicated—the end chromosomes in each preparation showing the line of division very clearly.

PHOTOS. 26 AND 27. A late anaphase, of second division. The eccentric chromosome can be identified at the left of each group and in both preparations shows a transverse furrow.

PHOTO. 28. Telophase of second spindle after the division of the eccentric chromosome which can be identified by its typical position outside the circle.

PHOTOS. 29, 30, AND 31. Another series of preparations demonstrating the division of the lagging chromosome. In Photo. 29 every chromosome is in evidence and the dyad form of the lagging chromosome is so marked it might almost be claimed as a demonstration of its division. In Photo. 30 the actual division of the lagging chromosome is shown and in Photo. 31 the complete separation of the daughter halves is demonstrated.

PHOTOS. 32 TO 39. Eight telophases of second spindles, all of them showing the eccentric chromosome ready to divide, though its division is retarded. In Photos. 35, 36, 38, and 39, the dyad form of the lagging chromosome is shown. Compare these preparations with telophases of first spindle shown in Photos. 6, 7, and 5. In Photos. 32, 33, and 34, we see the eccentric chromosome gradually turning to take the position necessary for its transverse division—this position is reached in Photo. 35, p. 298.

PHOTOS. 40, 41, AND 42. Telophases of second spindle showing the poles more widely separated, but in each preparation the lagging chromosome is attached to the poles by a delicate fiber, plainly visible in the preparation and clearly brought out in at least two of the photographs. The pointed ends of the lagging chromosome indicate that the daughter halves are being pulled apart, p. 298.

PHOTOS. 43 AND 44. Late telophases of second division—showing the poles widely separated and the eccentric chromosome with transverse furrow, lying midway between the poles. The telophase in Photo. 44 demonstrates that the lagging chromosome may divide at a very late stage of development. In this preparation the daughter cells, though still attached, plainly show the constriction of the cytoplasm which precedes cell division.

PHOTOS. 45 AND 46. In these preparations the final division of the lagging (eccentric) chromosome is demonstrated, p. 299.

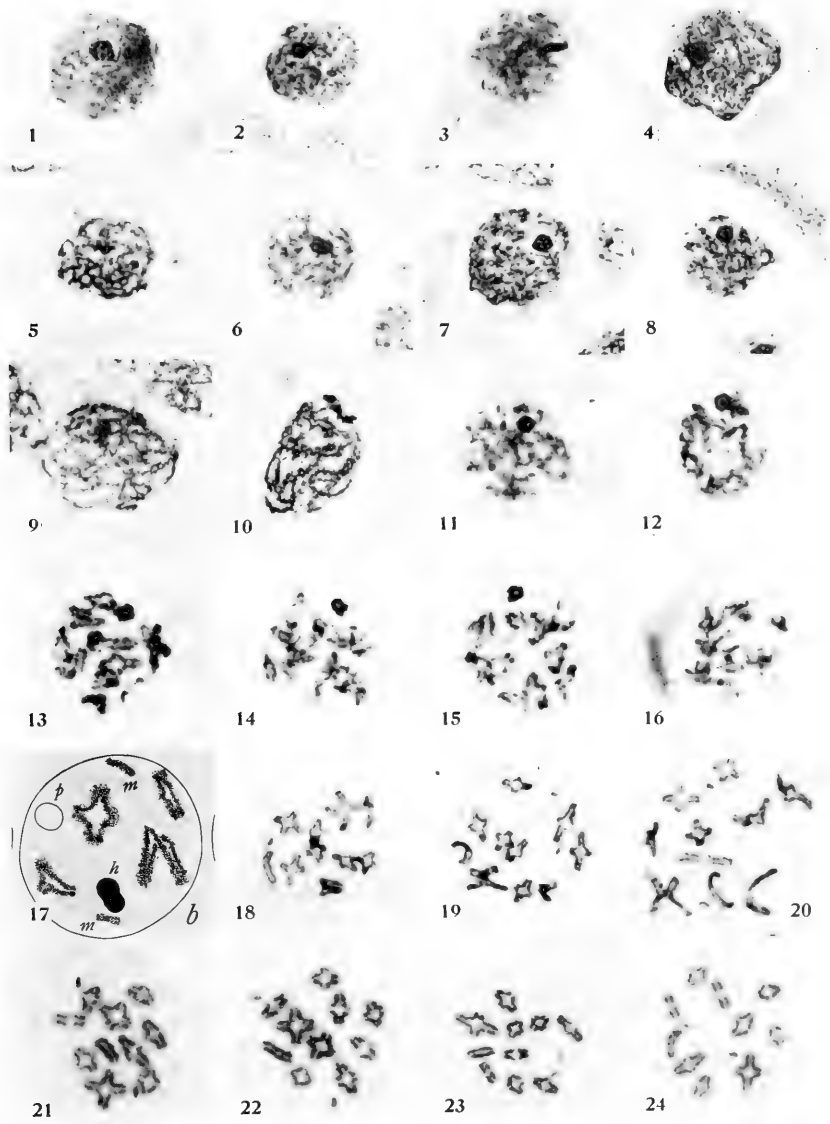
PHOTO. 47. Early spermatogonial prophase showing 20 large chromosomes and the two microchromosomes—one of these attached to a large chromosome. See text figure 4.

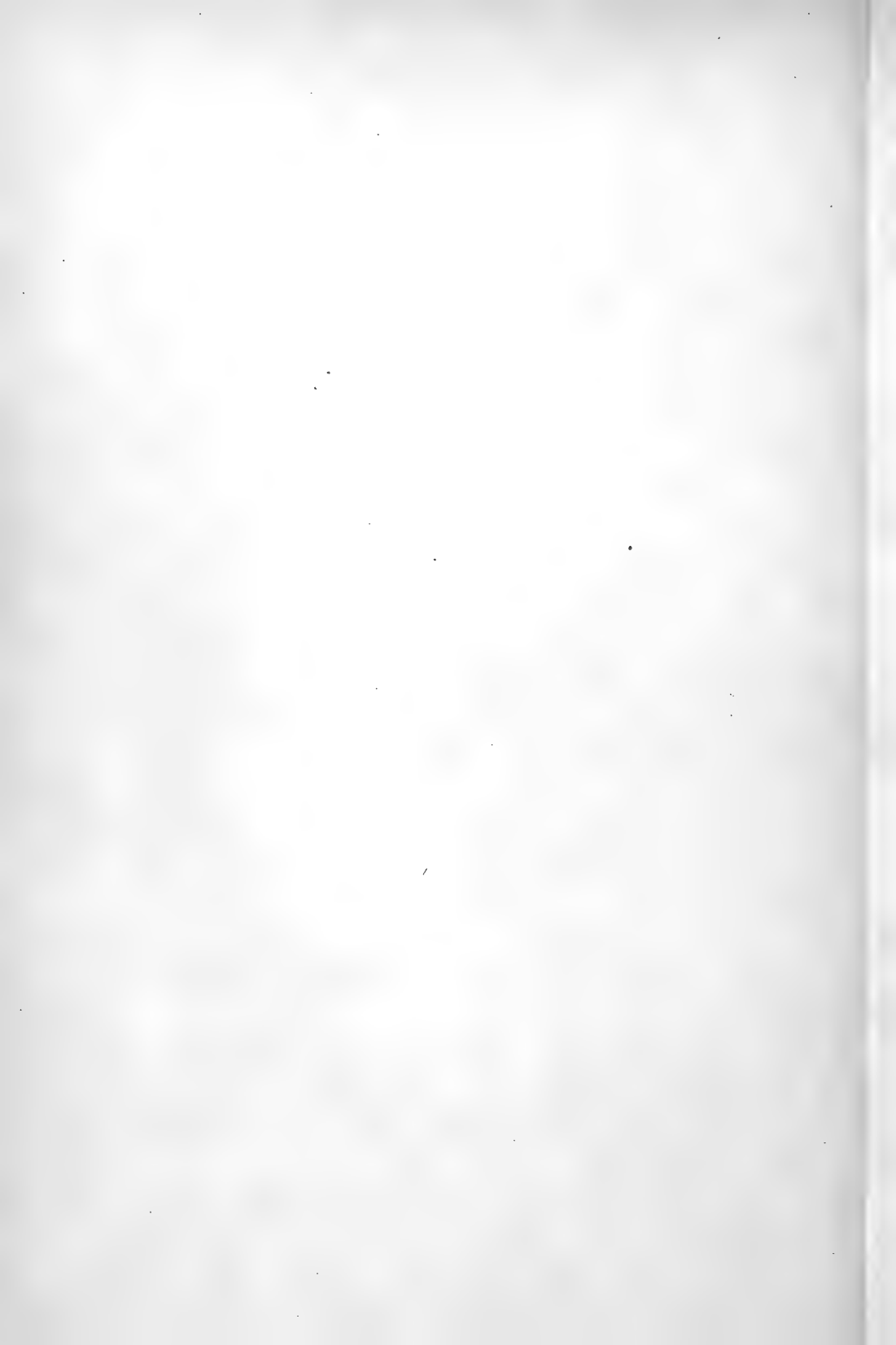
PHOTOS. 48 AND 49. A little later stage, each preparation showing 22 chromosomes.

PHOTO. 50. A still later stage where the chromosomes show the condensed form which precedes their division. At upper left hand center of group two chromosomes are in contact, attached almost at right angles. There are 22 chromosomes in this preparation.

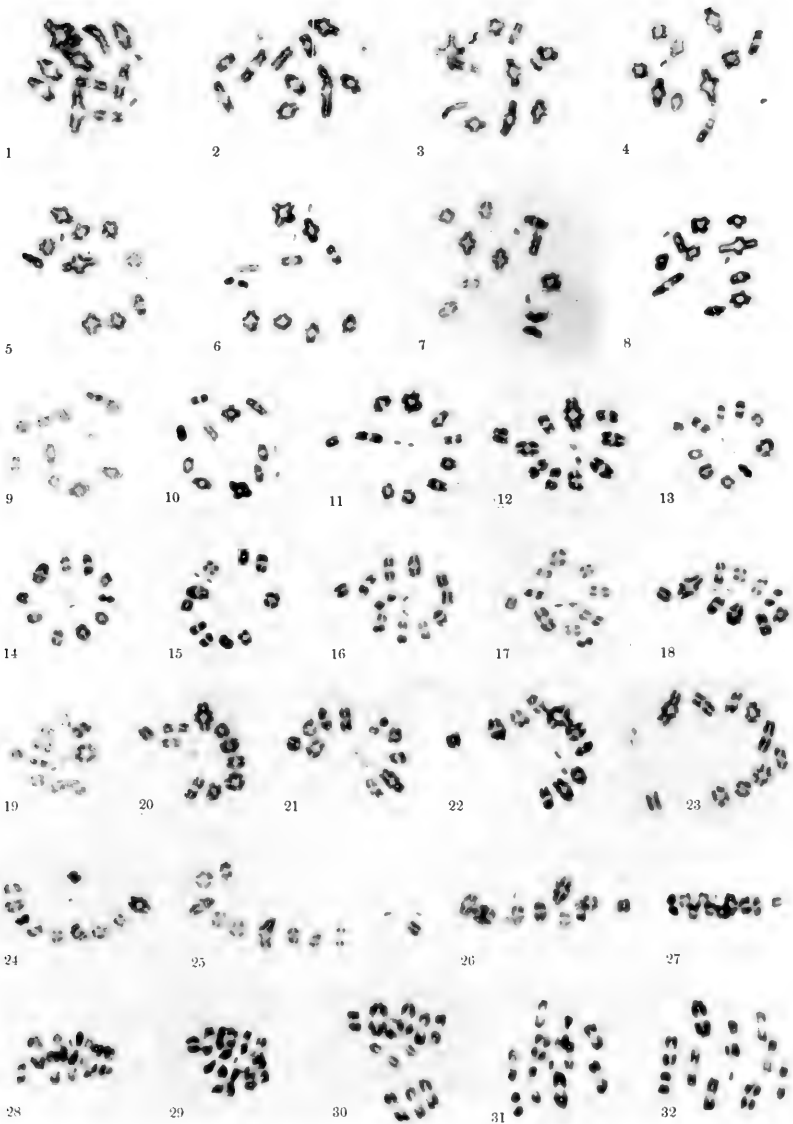
PHOTO. 51. Two spermatids each showing a well formed, deeply staining nucleolus.

FOOT & STROBELL PHOTOS.

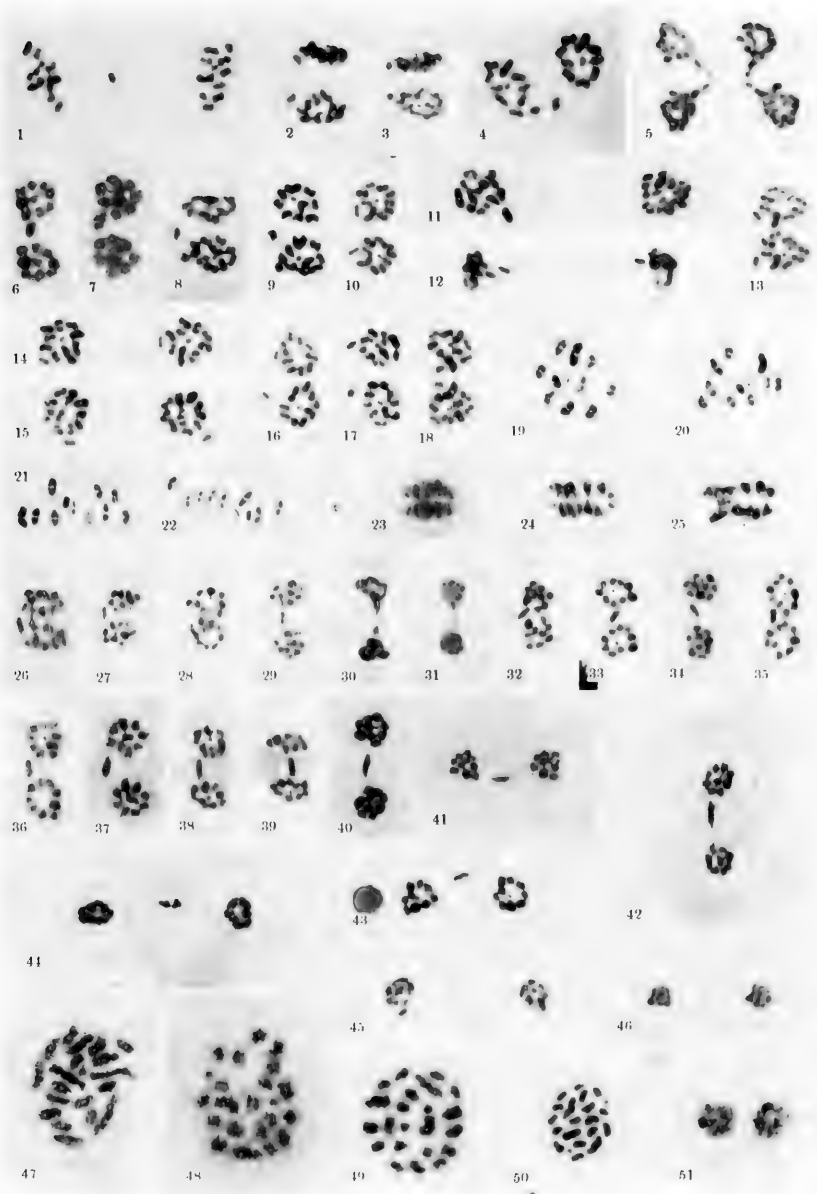




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THE CLOSING OF WOUNDS IN THE LARVAL NECTURUS.

BY

ALBERT C. EYCLESYMER.

From the Anatomical Laboratory of St. Louis University.

WITH 10 FIGURES.

While studying the normal development of *Necturus*, the writer frequently noted that accidental wounds closed with astonishing rapidity. These observations gave a starting point for the following experimental study.

The larvæ selected for the experiments were about 20-21 mm. in length. This stage was chosen because at this time the dermis is deeply pigmented over the dorsal surface and casual observations had shown that over this dark background certain light spots, presumably epidermal structures, moved toward the margin of the wounds. Each larva was placed in a solid watch glass containing a bed of cotton, in the meshes of which it soon became entangled and was thus held in given position. A small piece of skin about 2 mm. long and 1 mm. wide was then cut from the mid-dorsal region. In most of the experiments only the skin and subcutaneous tissues above the spinal cord were excised, yet in some, the spinal cord was more or less injured.

As soon as the excisions were made the larvæ were placed in separate dishes and examined either at short intervals, or continuously under the Zeiss binocular microscope with the No. a_3 objective and the No. 4 oculars. Under this high magnification, one could readily follow the movements of individual cells either remote from or at the margin of the cut. By this means it was found that the deeply pigmented dermal chromatophores remained practically stationary while large irregularly scattered epidermal cells moved toward the margins of the wound. By following the movements of these cells which the study of sections showed to be the unicellular gland cells, it was possible to determine the rate of movement of the epidermis over the dermis.

Since the rate of movement was practically the same in all the experiments, it will suffice to illustrate the process by a single series of diagrams which were taken at intervals from a single larva. On July 21, 9 a. m.,

a larva measuring 22 mm. was cut as above described and the movement of the gland cells followed. Their successive positions are represented in Figs. 1, 2, 3, and 4. In these diagrams the light colored oval area represents the excised portion. The positions of the epidermal gland cells are represented by the white spots while the dermal chromatophores are represented by the irregularly-branched black spots. Although the diagrams were made with the greatest care only approximate accuracy can be claimed.

If Fig. 1 be examined it will be seen that the gland cells have during the short interval, some 5 minutes, between the time of excision and placing beneath the microscope, moved over the margin of the cut toward the median line while the dermal chromatophores have remained practically stationary. Fig. 2 is a diagram from the same larva 30 minutes later. By comparing this with the condition represented in Fig. 1 it will be noted that while the gland cells pass toward the open part of the wound at approximately the same rate, there is considerable irregularity, *e. g.*, the cell lying between Nos. 1 and 2 has passed more rapidly toward the median line than either 1 or 2. The same is true of the gland cell lying just posterior to No. 8. Certain other peculiarities are noted, such as the appearance of gland cells Nos. 11 and 12, which were not visible in the preceding stage. By comparing any two gland cells bearing numerals, it will be seen, however, that they in general maintain the same relative distances from each other.

The position of the gland cells about 30 minutes later is shown in Fig. 3. At the time it was impossible to represent the gland cells which came into the field of the diagram from the periphery, consequently those along the margin of the diagram have been omitted. If the positions of cells Nos. 1 and 5 of Fig. 1 be compared with their position in Fig. 3, it will be seen that No. 5 has progressed more rapidly toward the center of the wound than has No. 1. In like manner No. 7 has passed more rapidly toward the center of the wound than has No. 2. While Nos. 4 and 10 have moved directly toward each other. Some of the cells have disappeared, as illustrated by the absence of one of the cells lying between Nos. 8 and 9, likewise, the cell lying just above No. 1. On the whole the diagram shows that the cells show a tendency to move toward the center of the wound and that in general they maintain their relative positions.

Fig. 4 represents the position of the gland cells 30 minutes later. In the diagram, the cells at the periphery have been omitted as in the preceding stage. The cells from the margin of the wound have continued

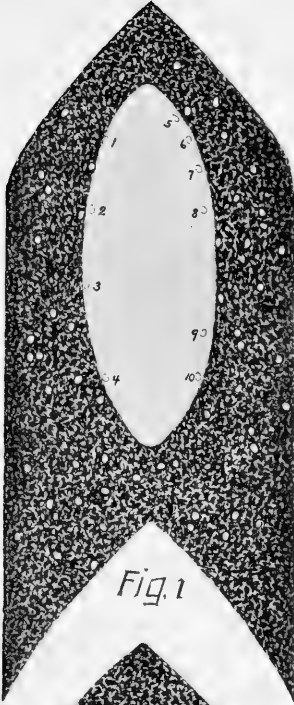


Fig. 1



Fig. 2



Fig. 3

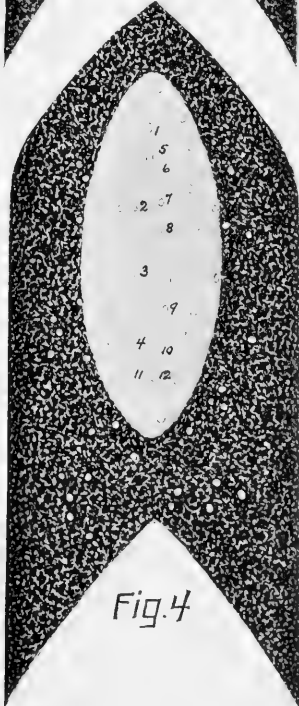


Fig. 4

their movement toward the central part of the wound until the margins are now in contact. Since no new features are observed beyond those mentioned in the above paragraphs, a detailed description is unnecessary. It should, however, be stated that the movement of the dermal chromatophores is practically imperceptible.

Twenty-four hours after the excision it was found, in all the larvæ excepting one, that the gland cells covering the wound have largely disappeared. The dermal chromatophores in some of the larvæ show a slight movement within the boundaries of the original cut, but on the whole there is little change in their position. July 26, 9 a. m., about 120 hours after cutting, the gland cells are still absent. The dermal chromatophores have extended within the original margin of the wound until one-half the area of the wound is covered. August 8, the epidermal glands are present in numbers and the chromatophores cover the entire wound.

The above study of the surface phenomena shows that in the brief interval of 90 minutes, the wound is completely closed when the temperature is about 18°C.

This closure is brought about through the movement of the epidermis over the dermis. The cells which participate extend over a considerable area, as shown by the shifting position of the gland cells in regions remote from the cut surface. Some of the gland cells disappear during the time the margins of the epidermis are approximating and after the closing of the wound they undergo such rapid degeneration that 24 hours later there are very few present.

After making as careful study of the surface phenomena as possible, this was supplemented by the study of serial sections, showing the various stages in the closing of the wounds. The larvæ were fixed in Zenker's fluid and were then stained *in toto* with Czoker's alum-cochineal, imbedded in paraffine, cut in serial sections and counter stained with Lyon blue.

The earliest stage studied in sections, is from a 22 mm. embryo about 5 minutes after cutting. In this particular larva, the epidermis, dermis and the greater portion of the spinal cord have been excised. As will be noted (Fig. 5) the wound has already filled with a coagulum made up of lymph, blood corpuscles and cellular fragments of the epidermis, dermis and spinal cord. The epidermis already shows a marked change in its character, in that it is much thickened around the margin of the wound. Often this margin is two or three times as thick as the normal. External to this thickened margin, the epidermis undergoes a gradual

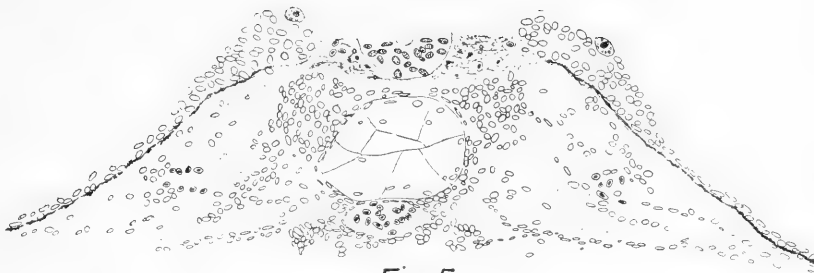


Fig. 5



Fig. 6

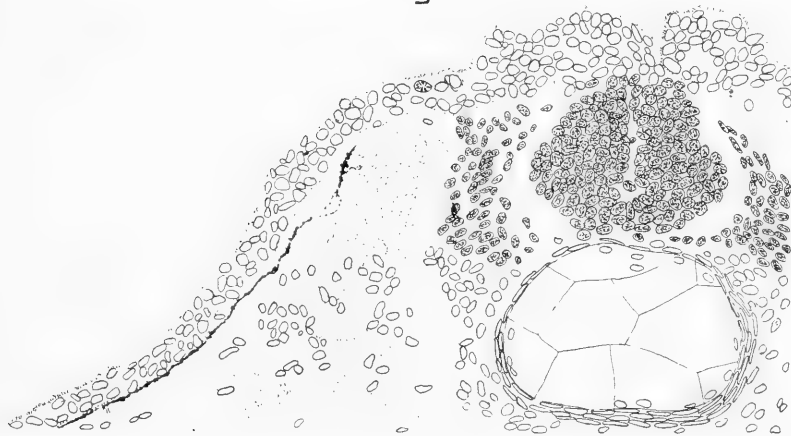


Fig. 7

T. S. Jones, del.

thinning until at the level of the outer margin of the myotomes it is but two layers thick at most, and often but a single layer. This thinning of the epidermis has been noted by Barfurth in *Rana* and *Siredon*. Not only is there a thinning of the epidermis through some sort of cell movement, but also the cells remaining as the single covering layer, often show a decided thinning through rotation and elongation.

The cells in the thickened margin show some peculiarities. They seem to be less compact than in the normal epidermis, their cell boundaries are not well defined and their cuticular margins are indistinct. The gland cells are readily distinguished in sections through their large size and numerous coarse yolk granules. The mitoses appear about as frequently as in the normal. The changes in the dermis are slight, the chromatophores show little if any movement toward the wound. It is impossible to say to what extent the dermal tissues are involved, such as blood-vessels, lymphatics, connective tissue, etc., owing to the fact that it is impossible to follow their changes closely enough in the sections.

The next section studied is from a larva at a stage corresponding to that shown in Fig. 3. At this time the margins of the original wound are clearly defined through the position of the chromatophores (Fig. 6). The epidermis has continued its movement toward the median line. The free margin of the epidermis is rounded and as in the preceding stages, is six or seven layers in thickness. Toward the periphery, at the outer margin of the myotome, the epidermis does not show the marked thinning which was observed in the preceding section, but is about the same thickness as in the normal larva, although there is no indication of a decided increase in the number of mitotic figures. The cell boundaries are poorly defined. The cuticle is more distinct than in the preceding stage and the gland cells are still present. The dermis shows no marked change either in movement of chromatophores or other structures.

A section representing the condition as observed some 90 minutes after cutting is shown in Fig. 7. The margins of the epidermis still show a decided thickening. In the dorso-lateral regions, the epidermis is not completely restored to its normal thickness. At this time the margins of the wound, are not only in close contact, but also have fused at their ventral edges. This fusion is brought about, so far as can be seen, by a simple coalescence of the cuticle-free margins of the thickened portions of the epidermis. The gland cells are present in the same relative numbers as in the preceding stage, as are also the mitotic figures. In some cases the closure of the wound does not take place precisely as outlined above, but a narrow band of cells extends between the thickened margins

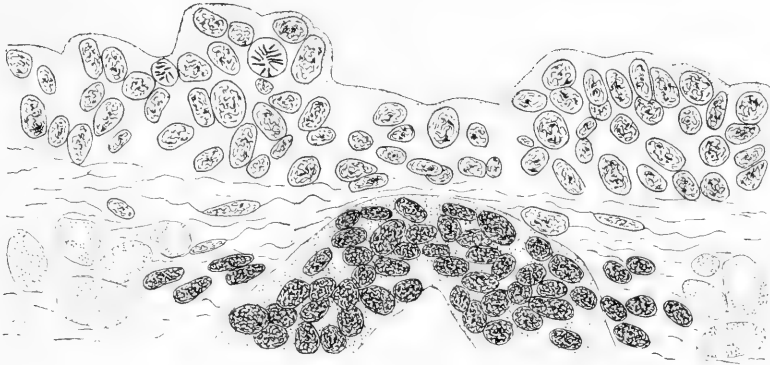


Fig.8

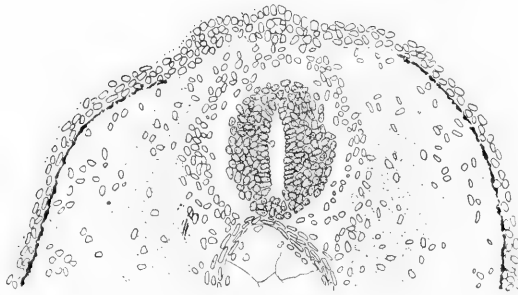


Fig.9

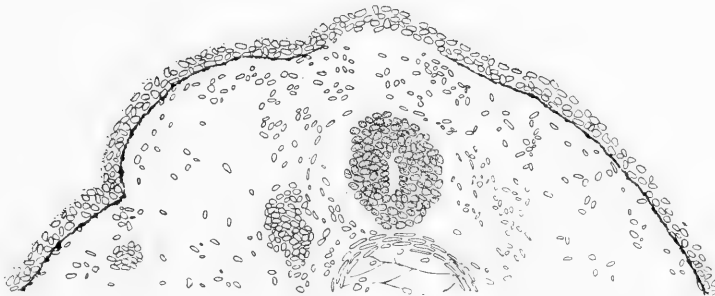


Fig.10

of the wound as shown in Fig. 8. At this time there are no features which enable us to say just where the line of coalescence exists. Mitoses are found frequently, but from several counts on both the normal and wounded at this stage one finds neither an increase or a decrease. A condition strikingly similar to this has been described and figured by Barfurth in *Rana*.

The condition 24 hours after excision is shown in Fig. 9. At this time one can still distinguish the original thickened margins of the epidermis, although they are becoming less and less pronounced. The cuticle has reappeared and is of the normal thickness and appearance. The gland cells in the epidermis are decidedly decreased in number; only a few being seen in the entire series of sections passing through the area of the wound. The mitotic figures are somewhat more numerous than in the normal condition. The dermal chromatophores show a migration toward the median line and the underlying connective tissue is present.

The section represented in Fig. 10 is from a larva 120 hours after excision. There is but the slightest indication of the line of fusion of the thickened margins of the wound. The epidermis being throughout nearly normal in appearance. The cuticle is as in the normal. One of the most striking features which has already been mentioned in the surface study is the almost entire absence of gland cells. Concerning their degeneration and regeneration but little is known and the material at hand does not permit a detailed description of these processes. All the structures have the appearance of the normal excepting the layer of chromatophores which has not extended completely over the original wounded surface.

RÉSUMÉ.

It may be remarked that wounds of the skin of the larval *Necturus* close so rapidly, that a wound 1 mm. in width closes in less than two hours. By raising the temperature some four degrees C., the rate of closing may be increased about one-third. Fraise states that in *Siredon*, a wound 2 mm. wide completely closes in five to six hours. In the same animal Barfurth found that when the tail was amputated the epithelium covered the wounded surface in about 75 minutes.

In the closing of the wound there is a movement of both the epidermis and dermis toward the center of the original wound. In this process the epidermis moves much more rapidly than the dermis as has been observed by Fraise, Peters, Barfurth, Loeb, Strong and others in various amphibia.

So far as can be gathered from all the known facts it would seem that cells become loosened as Barfurth maintains and those of the one side slowly move toward those of the opposite side until they meet. Barfurth seems to regard the movement as one of mass movement, as well as individual cells. He, however, explicitly states that the basal cells take no part in the movement. The process is somewhat different in *Necturus* since as pointed out, there is a participation in the extension of the epidermis by the basal cells in that they become greatly elongated in the direction of the extension of the epithelium.

All who have studied the healing of the wounds in the amphibia are agreed that the movement of the epidermis is not due to cell proliferation. Most hold that no mitotic figures can be found until many hours, even days have elapsed. My observations show that the mitotic figures are not increased, although present, until several hours after the margins of the wound have coalesced. At first, I was inclined to think that direct division played no part and had come to agree with Barfurth that it is extremely doubtful if any cases of direct division are present. My attention was again called to this subject through the work of Dr. C. M. Child, and after a careful re-examination of the sections I am inclined to believe that amitosis is a factor of considerable importance in the healing of wounds in the young *Necturus*.

The return of the cells to an indifferent character is another remarkable feature of the epidermis. It certainly appears, as Fraisse and Barfurth say, that the epidermis undergoes a degenerative change. The cell bridges, the cuticula and the glands disappear. All indicating that the epidermis returns to an indifferent condition. The cells become loosened from each other, take on amœboid movement and cover the wound, and when equilibrium is restored, the cells again become differentiated, giving rise to the structures which disappeared.

THE SKIN END-ORGANS OF THE TRIGEMINUS AND LATERALIS NERVES OF BDELLOSTOMA DOMBEYI.

BY

HOWARD AYERS AND JULIA WORTHINGTON.

WITH 10 FIGURES.

Owing to the noteworthy and rather complicated internal relationship of the *Trigeminus* and *Acustico-Lateralis* nerves of *Bdellostoma*, it has seemed best to publish a brief abstract of the results which a study of the peripheral terminations of these nerves has produced. The material on which the study is based consists of preparations made by the Golgi, the methylene blue, and the gold chloride methods, as well as sections stained with various coal-tar colors.

Stated briefly, the distribution of these nerves in the head of *Bdellostoma* is as follows: the skin territories of the dorsal part of the head cephalad of the brain, of the four tentacles, of the rim of the nasal tube, and of the upper part of the lip, are innervated by the *trigeminus*. The dorsal skin over the fore part of the brain and a little laterad of it is innervated by the *lateralis* posterior. The skin at the side of the head in front of the brain is within the territory belonging to both sets of nerves. Both *trigeminus* and *lateralis* fibers run to it through a common trunk, the *lateralis* component of which is the *lateralis* anterior.

The sense organs of the *trigeminus* described in this paper are those occurring in the tentacles and the rim of the nasal tube, while the sense organs of the *lateralis* are those found in the lateral line canals. Both kinds have been compared with those found in the territory supplied by both nerves. In this way we have sought to obtain a clear discrimination of the two kinds of organs.

The skin of *Bdellostoma* consists of two primary layers, a dermis and an epidermis. Essential agreement in structure with the skin of *Petromyzon* is the noteworthy fact.

The epidermis, when studied in sections stained with the usual general stains, such as hæmatoxylin or carmine, is seen to be composed of an

inner and an outer layer. The outer layer is, for the most part, composed of larger cells so arranged as to produce a smooth surface. As its cells die and fall away from the surface they are replaced from the inner layer. Numerous migrant mucous cells push their way from the basement membrane, lying on the dermis, outward, until they reach the surface where they break and discharge their contents upon the surface of the skin. With the exception of the mucous cells, the elements of the epidermis are relatively stationary.

Besides the more numerous polyhedral cells which make up the bulk of the epidermis, and omitting the mucous cells already mentioned, the other cell types met with are the sensory cells and supporting cells of the sense organs, the long club-shaped cells or palisade cells, and the undifferentiated cells from which all of these are produced. When, instead of using a hæmatoxylin or a carmine stain, some of the coal-tar colors are used, it is seen that the inner layer of the epidermis is itself sharply divided into two layers, a broad inner layer resting directly upon the basement membrane, and an outer layer of varying width. In material that has been hardened in chromic acid, this outer layer, the middle layer of the epidermis, does not stain with aniline green, Lyons blue, toluidin blue, or saffranin, but takes picric acid easily and holds it well. As the superficial layer, composed of larger cells, stains in the same color as the inner layer, it is thus shown that the division between outer and middle layers is not clean cut, as it looks in hæmatoxylin sections, for patches of yellow can be seen intruding into the outer layer, and lying between its cells. In Golgi sections a similar division is seen. The impregnation is extremely capricious in its action, here as elsewhere; sometimes the entire inner layer will be blackened, sometimes the entire middle layer. When this latter is the case, the blackening will sometimes extend far up into the outer layer, showing better than the color stains how deep the dovetailing of outer and middle layers really is.

THE TRIGEMINUS ENDINGS.

Free endings.—First and simplest of all are what appear to be free endings. The fine fibers of the nerve, on entering the epidermis, divide into still finer fibrils, some of even diameter, some beaded. Some of these fibrils take a comparatively straight course through the epidermis, and end in the outer layer, without forming nerve plates in connection with

any cell, and apparently without connecting with the epithelial cells in any way (Figs. 1 and 2); others, after running out a little way through the epidermis, turn and run parallel to the basement membrane, giving out in their course numerous fine fibrils that run, some mesad, some ectad. Some of these fine fibrils end in contact with cells, others, apparently, end free between the cells. Fig. 1, drawn from the common territory, also illustrates these relations as they exist in the tentacles.

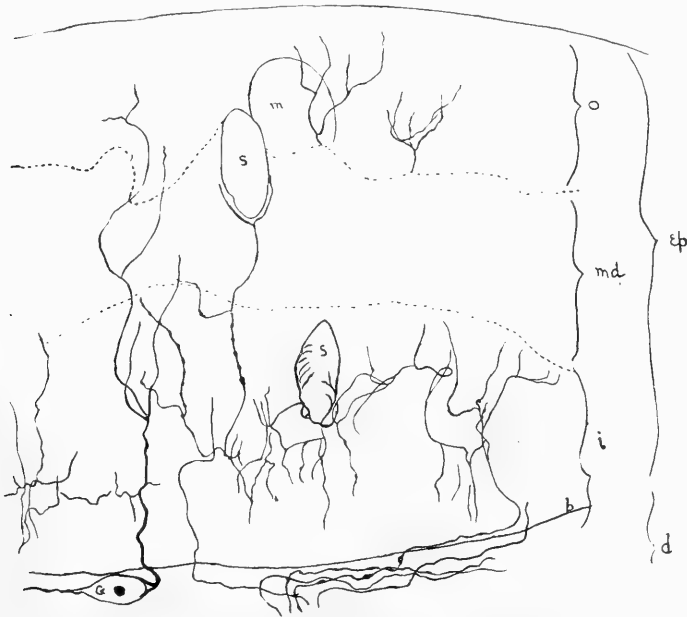


FIG. 1. Cross-section of skin of adult *Bdellostoma* through territory innervated by both *trigemini* and *lateralis* fibers. Golgi preparation. *b* = basement membrane, *d* = dermis, *ep* = epidermis, *G* = ganglion cell, *i* = inner layer of epidermis, *m* = mucous cell, *md* = middle layer of epidermis, *o* = outer layer of epidermis, *s* = sensory cell. $\times 153$.

Nerve end cells.—The single cells in connection with which *trigemini* fibers end, may be divided roughly into two classes, large cells and small cells. The small cells (Fig. 3) occur in either the inner or middle layers, and are very numerous. Many of them are oval, though some are conical as in Fig. 3, and taper to a sharp point at the apex. The nerve comes in contact with these cells at their proximal end; sometimes it forks (Fig. 3), sending a branch to lie against the cell on either side, sometimes it forms a small plate at the base of the cell, and sometimes it appears to penetrate

the cell, where it divides into numerous fine fibrils some of which lie parallel to each other (Figs. 1 and 3). The large cells (Fig. 4) are long

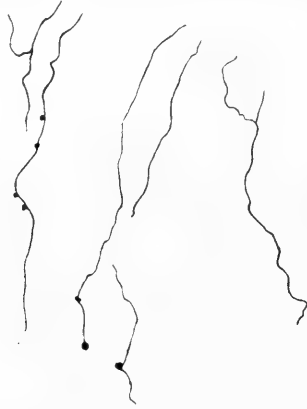


FIG. 2.



FIG. 3.

FIG. 4.

FIG. 2. Free nerve endings in the tentacle. $\times 533$.

FIG. 3. Small sensory cells in tentacle. *b* = basement membrane. 1 and 2 did not lie in the same field. $\times 533$.

FIG. 4. Large sensory cells in the tentacle. $\times 733$.

and narrow, club-shaped, generally wider at the proximal than at the distal end. They lie, either in the inner layer, or partly in the inner

layer, and partly in the middle one. The nerve enters them on the side, not far from the proximal end. There are comparatively few of these cells in the tentacles, and these few are comparatively small; in the skin of the head they are much larger and more numerous.

THE LATERALIS ENDINGS.

Although numerous investigators have searched for lateral line organs in *Bdellostoma*, both the organs and their canals have hitherto remained

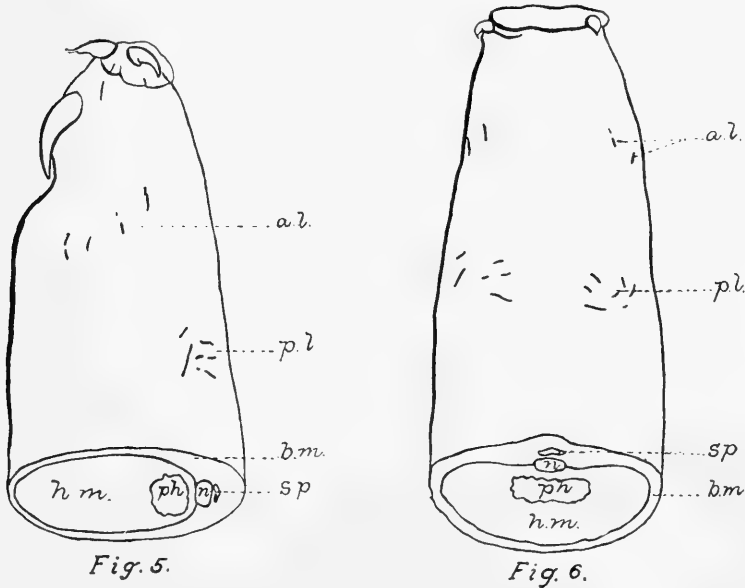


FIG. 5. Side view of head of young *Bdellostoma*, 11 inches long, preserved in chromic acid. *a. l.* = anterior lateral line canals, *b. m.* = body musculature, *h. m.* = head musculature, *n.* = notochord, *p. h.* = pharynx, *p. l.* = posterior lateral line canals, *sp.* = spinal cord. $\times 2\frac{2}{3}$.

FIG. 6. Dorsal view of same head. *a. l.* = anterior lateral line canals, *b. m.* = body musculature, *h. m.* = head musculature, *n.* = notochord, *ph.* = pharynx, *p. l.* = posterior lateral line canals, *sp.* = spinal cord. $\times 2\frac{2}{3}$.

undiscovered. The presence of lateral line canals in *Bdellostoma* is exceedingly interesting and noteworthy. The sense organs belonging to the Acustico-lateral system have been long recognized as present in the Petromyzonts, but in the *Bdellostomids* these structures have eluded all observers. Their existence in the latter forms is interesting and important because it demonstrates the presence of the surface organs from which

the ear sense organs arose, and shows them to be fully formed and functional in the lowest vertebrates above *Amphioxus*. The Acustico-lateral system of nerves and sense organs in *Bdellostoma* is as a whole not better developed, in comparison with the fishes (*e. g.*, Elasmobranchs), than is the Marsipobranch ear in comparison with the Elasmobranch ear. As we shall show, it stands as an intermediate stage between an earlier and still undescribed condition, and that found in higher forms. The facts relative to the Acustico-lateral system of *Bdellostoma* constitute another

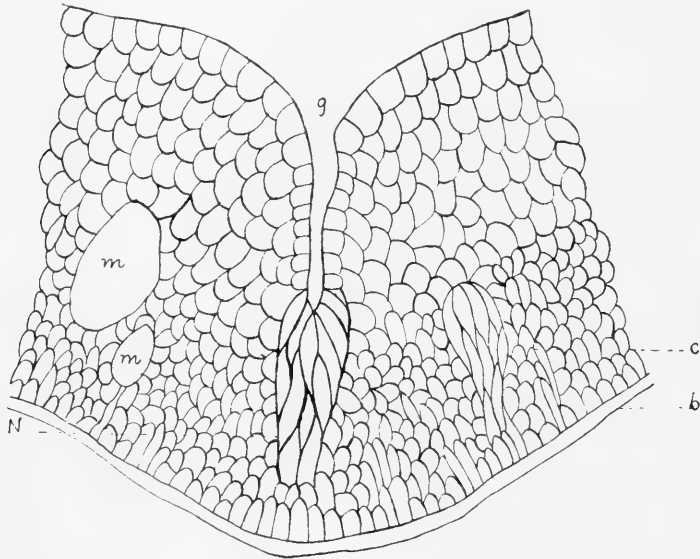


FIG. 7. Cross-section of epidermis of young *Bdellostoma*, 11 inches long, through lateral line canal. Preserved in formalin. *b* = basement membrane, *c* = spindle-cell neuromast at side of dermal groove, *g* = lateral line canal, *m* = mucous cell, *N* = central spindle-cell neuromast ridge. $\times 333$.

and an important contribution to the growing evidence that the Marsipobranchs are not degenerate forms, but occupy a place in the direct line of descent of the phylum from the ancestral form.

In *Bdellostoma* the canals of the lateral line system are certainly difficult to find largely because they are exceedingly small, and the surface indications are so faint that even in heads preserved in chromic acid, only an eye that knows exactly where to look and what to expect can detect them in the full-grown adult. In the young hagfish not more than eleven or twelve inches long, the chromic acid treatment shows them much more plainly.

The canals are arranged in two groups, an anterior and a posterior one, in the dorso-lateral territory of the head, corresponding to the anterior and posterior *lateralis* nerves by which they are innervated. Figs. 5 and 6 show the position of these groups on the head. The anterior group of canals is composed of four, occasionally three or five short canals, nearly equidistant from each other. This group of canals is located on the side of the head in front of the eye, of its side of the body. The cephalic end of the most ventral canal is slightly caudad of the hind end of the most dorsal one (Fig. 5). The posterior group lies on the dorsal surface of the head and consists of two divisions; the three (occasionally two) inner canals run meso-laterad, and the outer ones run at a slight angle to the long axis of the body (Fig. 6).

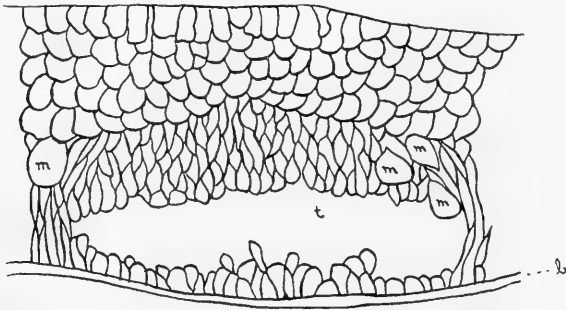


FIG. 8. Cross-section of epidermis of young *Bdellostoma*, 11 inches long, close to lateral line canal. Preserved in chromic acid. *b* = basement membrane, *m* = mucous cell, *t* tube above dermal groove. $\times 333$.

Cross-sections through the canals show an interesting and rather peculiar state of affairs, and also a condition that differs according to the original preservative used. Fig. 7 is a cross-section through one of the dorsal canals of a young hagfish about eleven inches long. The head from which it was taken was preserved in formalin. The canal itself (Fig. 7, *g*) is seen as a deep, narrow groove in the outer layer of the epidermis. In the figure its bottom is formed by the outer ends of a group of long, slender spindle cells (Fig. 7, *c*), grouped to form a fusiform body. In studying serial sections, however, this group of spindle-shaped cells is found to extend along the entire bottom of the canal. In some sections it extends down to the basement membrane, in others only about one-half the distance. There is a distinct line of cleavage down the center of this ridge of spindle cells, so that in some sections it sep-

arates in halves, the cavity of the canal being continuous with the break in the ridge. Beneath the canal and ridge is a very decided depression of the basement membrane, so that the entire apparatus, canal and ridge together, rests in a groove of the dermis (Fig. 10). This groove does not terminate with the canal above it, but extends a short distance, sometimes thirty microns, sometimes one hundred and thirty, beyond it at each end. Moreover, where the surface canal is interrupted (Figs. 5 and 6), sections show the groove beneath to be continuous.

At one side of the dermal groove in Fig. 7 is seen a second body of spindle-shaped cells, inclined towards the canal. These side bundles are formed of a row of club-shaped bodies; sometimes they are continuous

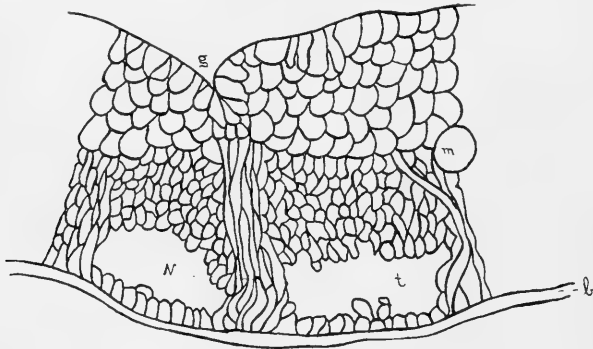


FIG. 9. Cross-section of epidermis of young *Bdellostoma*, 11 inches long, through lateral line canal. Preserved in chromic acid. *b* = basement membrane, *g* = lateral line canal, *m* = mucous cell, *N* = spindle-cell ridge, *t* = tube beneath canal and above dermal groove. $\times 333$.

ridges, shorter, however, than the central ridge. Like it they have a definite line of cleavage down the center, and are sometimes connected by a branch opening directly with the canal.

Similar club-shaped bundles of spindle cells are also found outside of the dermal groove. They are numerous in the territory of the *lateralis* anterior, but much less so in that of the *lateralis* posterior. Nerve fibers run into the bundles, and they are undoubtedly genuine neuromasts.

In addition to these neuromasts, single sensory cells are found in the territory of the dermal groove, (Fig. 10, *s*). They are larger than the epithelial cells about them, are bluntly conical in shape, and do not stain readily. These sense cells are not abundant, but a more successful method of staining may show them to be more numerous than they

appear at present. They have been found outside of the dermal groove as well as within it.

When instead of skin preserved in formalin, skin of a young hagfish that has been preserved in chromic acid is used, startlingly different results are obtained. The essential features, the deep surface canal of the epidermis, the spindle-celled ridge, the dermal groove, are all present; but in addition to these the chromic acid causes the inner epidermal layer to separate into two parts along what appears to be a definite line of weakness, and thus forms closed tubes that appear between the dermal groove

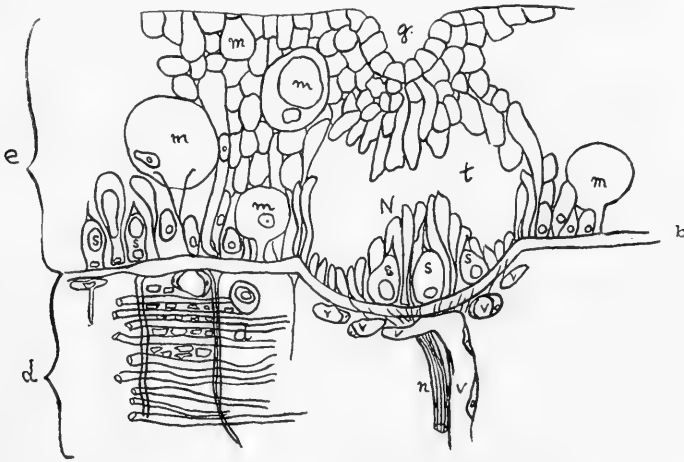


FIG. 10. Cross-section of skin through lateral line canal of young *Bdellostoma*, 11 inches long, preserved in chromic acid. *b* = basement membrane, *d* = dermis, *e* = epidermis, *g* = lateral line canal, *m* = mucous cell, *n* = nerve bundle, *N* = spindle-cell ridge (broken across), *s* = single sensory cell, *t* = tube beneath canal, *v* = blood-vessel. = 153.

and the superficial canal (Figs. 8, 9, and 10, *t*). The closed tubes are as long as the dermal grooves, and consequently longer than the surface canals. Fig. 8 is a typical section of one of these tubes just before its end, after passing the end of the canal, and this same appearance is also found in the territory of the closed tube in the inter-spaces shown in Figs. 5 and 6 in which the surface canal is interrupted. The closed tube is, for the most part, separated by only a single layer of small cells from the basement membrane. Above the lumen of the tube are several layers of small cells before the large celled layer of the epidermis is reached. The sides of the tube are usually bounded by long slender cells, and its roof, in this section, contains several mucous cells.

In chromic acid material the canal has sometimes the appearance of a pinching in of the epithelium, as in Fig. 9, and at others the appearance of a well-formed groove as in Fig. 10; in formalin sections it is ordinarily deeper and narrower, as in Fig. 7. The central ridge of spindle cells has the same relation to the canal in chromic acid sections as is shown in those preserved in formalin, but in Fig. 9, where the sides of the canal have been pinched together, this relation is not so easily recognizable.

In some sections the ridge runs only as far as the lumen (Fig. 10), and in others, as in Fig. 9, it crosses the lumen to dovetail into long slender cells of the same kind resting on the basement membrane, thus bisecting the tube. This bundle of spindle cells has, in cross-section, the appearance of a plug, and the line of cleavage down the center is occasionally well marked.

There is still a third typical cross-section of the tube (Fig. 10). In it the spindle-celled ridge has apparently been torn in two across the middle along a plane parallel with the surface, leaving a decided ridge rising from the floor of the tube. In this lower ridge are found several single sensory cells (Fig. 10, *s*).

From the preceding statements two facts stand out prominently. The first is the embryonic condition of the canals as compared with the typical canals of higher vertebrates. In *Bdellostoma* the canals remain throughout life entirely within the epidermis. At no stage of growth do they acquire a dermal sheath. They thus represent in their adult condition a stage of growth present only in the embryos of higher vertebrates. The second is the relatively low stage of differentiation of the sense organs. The dermis is depressed underneath the canals, but the edges of the grooves thus formed do not grow upward to enclose the epithelial canal. The dermal groove is never deep enough to enclose the sense organ.

CINCINNATI, O., April, 1907.

THE CORTEX OF THE BRAIN IN THE HUMAN EMBRYO
DURING THE FOURTH MONTH WITH SPECIAL REF-
ERENCE TO THE SO-CALLED "PAPILLÆ OF RETZIUS."

BY

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WITH 6 FIGURES.

The observations reported in the following paper conclusively show that the cortical granulations, or papillæ of Retzius, caused by the fungiform arrangement of the cells of the pyramidal layer, and commonly found in human embryos between 11 and 14 cm. long, constitute an abnormal condition, which is produced either by intrauterine or postpartum maceration. It is pointed out that of two human brains of the same age one may have cortical papillæ while in the other they may be absent. Furthermore, it is shown in pig brains, where cortical papillæ are not normally present, that it is possible by experimental methods to produce a fungiform clumping of the cortical cells that exactly duplicates the condition seen in human brains.

Attention was first called by Retzius, 95, p. 17, to the fact that human brains, usually of the fourth month and more rarely of the fifth month, possess a fine granulated character perceptible through the smooth surface of the cortex, and in places where the thin superficial layer (Randschicht of His) had been torn off they appear correspondingly granulated or covered with rounded elevations. Microscopical examination of sections of these regions revealed the fact that the granulated character was due to an unequal growth of the pyramidal cell layer, which projected in rounded elevations, the spaces between which were filled in by the superficial or moleculare layer, so that the surface of the brain remained smooth. Retzius considered the possibility of this granulation formation being a manifestation of some pathological process, such as is commonly associated with abortion, *e. g.*, syphilis. He was, however, more inclined to believe it a normal condition due to a transitory exuberant growth of the pyramidal cell layer, the surface irregularities caused thereby being

smoothed out later by the development of the adjacent layers. Shortly after this Hochstetter, 98, p. 5, briefly refers to the granulations of the pyramidal cell layer described by Retzius. He confirms their presence in poorly preserved brains, and designates this appearance as a decomposition phenomenon, without giving any further evidence.

Two years later His, 00, not having noticed the observations of Retzius, described independently the same peculiar granular or wart-like character of the pyramidal layer in embryo brains of the fourth month; and again in his last work His, 04, he describes at some length this appearance under the title "Die Retziusschen Warzchen." One of his illustrations is reproduced in Fig. 1. Though in his discussion he admits that it is

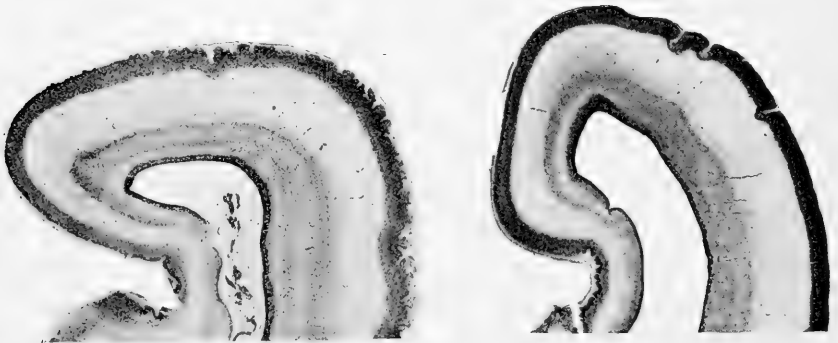


FIG. 1.

FIG. 2.

FIG. 1. Section through the occipital lobe of the brain of a human embryo, 12 cm. long (end of 4th month), showing the irregularities of the pyramidal zone caused by the fungiform clumping of the cortical cells, the so-called papillæ of Retzius. Taken from His, 04, Fig. 75.

FIG. 2. Section from the brain of a human embryo of about the same age and taken from the same place as shown in Fig. 1. Here the pyramidal zone consists of a compact layer with parallel borders, which, with the exception of three transitory fissures presents a perfectly smooth outer surface, and shows no trace of the Retzius papillæ.

still an open question; yet he is apparently inclined to consider the papillæ as normal, and does not hesitate to discard the possibility referred to by Retzius of their being pathological, on the ground that several of his specimens, which showed characteristic cortical papillæ, came from healthy individuals who had committed suicide, and the fetuses themselves appeared normal. He also argues that if it were a post-mortem alteration, associated with the swelling of the tissues, then the superficial layer would also present an irregular surface, which is not the case.

Neither His nor Retzius accounted for the fact that this phenomenon

has not been observed in brains of other animals. With this in mind the writer decided to make the test on some other mammal, fresh embryos of which could be easily obtained at all ages, and where control experiments could be carried out on embryos of the same litter. The pig was selected, and an examination was made of brains of embryo pigs measuring from 10 to 14 cm. long, which is a period that corresponds to and fully covers the time of appearance of cortical papillæ in the human brain.¹

It was found that the pyramidal layer in the pig does not exhibit any cortical papillæ when carefully preserved; but has always a smooth, regular surface, the only indentations being those corresponding to the beginning fissures, which make their appearance in specimens between 12 and 13 cm. long. A photograph of a section of a normal brain of a 11.5 cm. pig is shown in Fig. 3.

Having found that cortical papillæ are not normally present in the pig, the next step was to see whether they could not be produced artificially, and preferably under conditions which might be probable in case of human material. Two possibilities suggested themselves as etiological factors; in the first place, maceration of the specimen before it was put into the preserving fluid, and secondly imperfect penetration of the preserving fluid. Under maceration we should have to consider both post-partum and intrauterine maceration. The latter might be brought about, for instance, by disease of, or abnormal attachment of the placenta with consequent disturbance of circulation, and perhaps death of the foetus some days before abortion. The second condition, faulty penetration of the fixative, might be present in brains of this size were the fixative not injected through the arteries or the brain coverings not immediately opened up so as to permit the direct action of the fluid on the brain itself.

The following experiments were carried out with the idea of imitating these two conditions; on the one hand, for obtaining imperfect penetration

¹ The fact that the cortical papillæ are usually limited to the fourth month may perhaps be explained as follows: Up to that time the brain wall is relatively thin and uniform in structure, so that deformities then take the form of complete foldings of the wall. In specimens of the fourth month the wall is sufficiently thick to prevent foldings of the entire wall, and expansion and shrinkage express themselves in a readjustment of its constituent parts, some parts being more affected than others. In older specimens such a readjustment is prevented by the development of the cell processes and the supporting framework of neuroglia, resulting in a structure sufficiently firm to preserve its form in the fixative, and consequently no more papillæ or artificial fissures are found.

of the fixing fluid, the brain coverings were not removed until the specimen was ready for embedding, and on the other hand, the maceration was produced either by keeping the specimens dry and exposed to the air long enough for them to macerate in their own fluids before they came into the fixative, or in other cases by putting the brains directly into normal salt solution for varying lengths of time. In human material His found the cortical papillæ most marked in material hardened first in formalin and then immersed for several days in Müller's solution. So the same method of fixation was adopted in the experiments, the details of which are as follows:

A. Maceration Followed by Imperfect Fixation.

- A1. Maceration in own fluids (11, 12, and 14 cm. pigs).
 Embryos left exposed to air, 18 hours.
 Embryos placed in formalin, 10%, 48 hours.
 Embryos placed in Müller's solution, 4 days.
 Washed, brought into alcohol, and then the brain coverings were removed and the brain imbedded and cut in paraffin.
- A2. Maceration in normal salt solution (12 cm. pigs).
 Embryos placed in salt solution, 17 and 48 hours.
 Embryos placed in formalin, 10%, 48 hours.
 Embryos placed in Müller's solution, 4 days.
 Washed, brought into alcohol, and brain removed as above and the brain then sectioned in celloidin and paraffin.

In these specimens, in which the brain coverings were left intact throughout the period of fixation, no cortical papillæ were found. Embryos of different sizes were tried (A1) for the purpose of covering the whole period favorable to the formation of the papillæ. The poor preservation of the tissues manifested itself by a varying degree of fragmentation of the sections, particularly of the deeper parts. The sections presented a shredded appearance which varied from minute forking clefts between small clumps of cells and between fiber bundles, up to large irregular cracks splitting the different layers of the brain wall. This condition was found both in material that was cut in paraffin and in that cut in celloidin, but it was more marked in specimens that had been macerated in salt solution 17 hours, and still more marked in those macerated 48 hours. Otherwise the general topography of the sections and the arrangement of the layers was fairly well preserved. The minute clefts between the cells of the pyramidal layer gave a slightly ragged appearance to the surface

of that layer, but it was nothing that approached the fungiform arrangement seen in the Retzius papillæ. As can be seen in normal specimens at this time, the pyramidal layer is split by a line of scanty nuclei into a



FIG. 3. Section from a well-preserved brain of a pig embryo, 11.5 cm. long. This section shows that normally, in the pig brain of this age, the pyramidal zone presents a uniformly smooth outer surface. This brain, while still warm, was preserved in a chrome-acetic mixture.

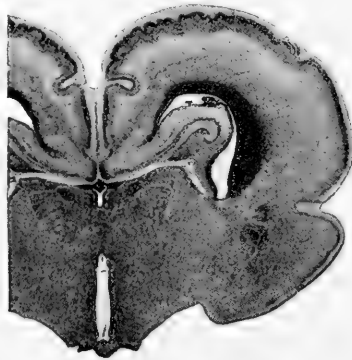


FIG. 4. Section from a macerated brain of a pig embryo, 11.5 cm. long. The brain was kept in normal salt solution 48 hours and then preserved in formalin followed by Müller's solution. The section shows distinct fungiform clumping of the cortical cells and characteristic Retzius papillæ. The same specimen is shown under higher power in Fig. 6.

more superficial thicker subdivision, the pyramidal cells proper, and a deeper subdivision which is to form the layer of polygonal cells. This stratification was preserved in the experimental material. Another feature of importance was the absence of the so-called transitory fissures.

B. Maceration Followed by Good Fixation.

- B1. Maceration in normal salt solution (11.5 cm. pigs)
- (a) Fresh brain placed in salt solution, 28 hours.
Hardened in chrome-acetic solution, 48 hours.
Washed, dehydrated and sectioned in celloidin.
 - (b) (Figs. 4 and 6) Fresh brain placed in salt solution, 48 hours.
Hardened in formalin 10%, 24 hours.
Secondary fixation in Müller's solution, 4 days.
Washed, dehydrated and sectioned in celloidin.
- B2. Maceration in its own fluids (11.5 cm. pig) see Fig. 5.
Embryo left exposed to air, 48 hours.
Brain removed and kept in formalin, 48 hours.
Secondary fixation in Müller's solution, 4 days.
Washed, dehydrated and sectioned in celloidin.

The sections of the specimens macerated in salt solution (B1, a and b) show fairly good preservation of the deeper lying parts, there is almost no shredding of the tissue like that seen in the specimens in which the penetration of the fixing fluids was hindered by the brain coverings. The pyramidal layer of the cortex, however, is found to be thrown into irregular folds, accompanied by a fungiform clumping of its constituent cells. This appearance is present in both specimens, but is more marked in the specimen (b) macerated 48 hours. A section of this was photographed and is reproduced in Fig. 6. The resemblance is close to the description given by His and Retzius of the cortical papillæ in the human embryo. It has the same smooth-surfaced superficial layer, which dips down between the papillæ of the subjacent pyramidal layer. In some places these incisures cut off small irregular islands of pyramidal cells. The inner surface of the pyramidal layer does not have these sharp notches, but runs across the section in an irregular wavy ill-defined line. In addition to the fungiform clumping of the cortical cells, in some of the sections the so-called transitory fissures are found. These dip sharply inward and invade in some cases more than one-third of the thickness of the brain wall. In the formation of these, the superficial layer is partially folded in with a corresponding cleft on the surface of the brain, which is not the case with the cortical papillæ. It may be noted that the artificial character of transitory fissures has been well established by Hochstetter, 98, and Mall, 03, the latter having examined over fifty embryos and found that according to the effect of various dissociating influences he could obtain macera-

tion in all stages, from simple folding of the brain wall up to conversion of the entire central nervous system into a pulpy mass. Evidently cortical papillæ and transitory fissures, though differing in character, have a similar etiology; as in the above experiment we have both, artificially produced in the same brain under known conditions. The interesting fact should be noted that though the two formations may occur in the same brain, and may closely adjoin each other, yet they do not occur together at the same place; that is to say, one does not find a fungiform grouping



FIG. 5.



FIG. 6.

FIG. 5. Section of the brain of a pig embryo, 11.5 cm. long. The embryo was left exposed to the air, and the brain allowed to macerate in its own fluid 48 hours. The brain was then removed and preserved in formalin followed by Müller's solution. In this section the fungiform clumping involves only the outer part of the pyramidal zone, and in this respect closely resembles the condition seen in Fig. 1.

FIG. 6. Section of same specimen shown in Fig. 4. The maceration here is more advanced than that seen in Fig. 5. The fungiform clumping involves the whole thickness of the pyramidal zone, both the inner and outer surfaces of which are thrown into coarse irregular folds.

of the cells that lie in the cleft of a transitory fissure. Either process seems sufficient to satisfy the space demand.

Sections from the specimen macerated in its own fluids (B2), see Fig. 5, differ from those macerated directly in salt solution in that the fungiform arrangement of the cortical cells involves only the more superficial part of the pyramidal layer. Instead of foldings of the whole layer, such as is seen in Fig. 6, we have here only a granulated or fungiform surface; and this duplicates almost exactly the condition found in the embryo P1

of His, which he pictures in Figs. 75, 77, 98 and 99. It shows that it is possible by different methods of maceration to produce experimentally typical cortical papillæ in brains where they are not normally present.

CONCLUSION.

The comparison of Figs. 1 and 2, one with, and one without cortical papillæ, suggests the probability of the abnormal character of the papillæ. One could still perhaps raise the objection that they may be normal, but very transitory, and that the two sections do not quite represent the same stage of development, so that in Fig. 2 the papillæ have either already disappeared or have not yet developed. This objection, however, can no longer be considered in face of the fact that in pigs, where one is able to secure specimens in exactly the same stage of development, it is possible, as has been shown above, to produce the papillæ by means of maceration, and furthermore to control their size and character by varying the degree and method of maceration.

From the experience derived from the above experiments, as regards conditions which predispose to artificial fissures of the cortex and deformities of its constituent cell layers, it becomes evident that embryo brains, which are intended for general morphological study, should, up until the time of completion of the principal fissures, be hardened *in situ* without disturbing the brain coverings. If the brain of a human embryo fresh from the uterus is uncovered or completely removed, and then immediately immersed in formalin or other fixative, it will not necessarily be free from abnormal fissures, etc. The framework of the brain wall up to that time is by no means firm, and it must be also remembered that it may already have been softened by maceration in the uterus. Thus in such a case, and much more so in the embryos that do not reach the hardening fluid so promptly, it is essential that the brain coverings should be left intact, that they may serve as a support to the brain during the process of fixation. Imperfect penetration of the preserving fluid is to be obviated by injecting it through the blood-vascular system.

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THE SPERMATOGENESIS OF BUFO LENTIGINOSUS.

BY

HELEN DEAN KING, PH. D.

WITH 3 PLATES AND 2 DIAGRAMS IN THE TEXT.

Although the spermatocytes of amphibians seem to be exceptionally favorable material for a study of maturation phenomena, there has been considerable controversy among the workers on amphibian spermatogenesis regarding the character of the maturation divisions. Several investigators, among whom may be mentioned Meves, McGregor, and Eisen, maintain that both of the maturation divisions are longitudinal; Montgomery and vom Rath, on the other hand, claim that only one of the divisions is longitudinal, the other being a reduction division in the Weismannian sense. It seems necessary, therefore, that the behavior of the chromatin in the germ-cells of more species of amphibians should be studied in order that this point may be definitely determined.

With the exception of vom Rath (46, 47), Bühler (7), and Broman (6), the investigators who have recently studied the spermatogenesis of amphibians have worked on various species of the Urodela. Vom Rath's study of *Rana* was very fragmentary and his results have been severely criticised; the researches of Bühler on *Bufo vulgaris*, and of Broman on *Bombinator igneus*, were confined to the development of the spermatid into the spermatozoön. The spermatogenesis of the Anura is, therefore, practically an unworked field. The present paper records investigations on the common toad, *Bufo lentiginosus*, which form the starting point for a study of the chromatin relations in the germ-cells of various species of amphibians. It is only by a comparative study of many forms that one can safely draw conclusions for an entire class.

MATERIAL AND METHOD.

Testes of adult toads killed at various times from April until September, and also testes of young toads with a body length of from 2 cm. to 6 cm. were used for these investigations. In both kinds of material like processes of development were found to be taking place, and it is evident that the spermatozoa formed anew each year in the adult undergo

a course of development similar to that of the spermatozoa which are first formed in the young.

The testes of the adult toad are cylindrical bodies lying directly in front of the kidneys. They measure from 10 mm. to 12 mm. in length and from 2 mm. to 3 mm. in width, the difference in size depending doubtless on the age of the toad. At the anterior end of each testis, and continuous with it, is a small, rounded structure, the so-called "Bidder's organ." This body is probably a rudimentary ovary, as several investigators have maintained, and it has seemingly nothing to do with the development of the spermatozoa.

Each testis is divided into a number of compartments, or follicles, separated by comparatively thick walls which contain numerous follicle cells. Each follicle is, in turn, divided into several smaller compartments, or cysts, which are separated from each other by much thinner walls. As a rule, all of the cells in a cyst are in approximately the same stage of development; but a single follicle may contain both spermatogonia and maturing spermatids. A transverse section of the testis, therefore, shows practically all stages in the development of the spermatozoa. As might be expected, testes of adult toads killed soon after the end of the breeding season contain large numbers of dividing spermatogonia; while the testes of toads killed in August or September contain relatively more spermatocytes and spermatids.

The structure of the testis of a young toad is similar to that of the adult except that there are fewer follicles and cysts. Until the toad has attained a body length of about 2 cm. the testes contain nothing but spermatogonia surrounded by follicle cells. After this time some of the cells evidently develop much faster than others, as spermatids can always be found among the spermatogonia and spermatocytes in the testes of toads 3 cm. long. When the young toad is about 5 cm. in length sections of the testes, except for their smaller size, cannot be distinguished from those of an adult killed in the early autumn.

Various fluids were used for fixation of the material, by far the most satisfactory being Flemming's solution (strong formula). Corrosive-acetic, which gives exceedingly good preparations of the egg, is but an indifferent fixing fluid for the testes as is also Gilson's mercurio-nitric solution. As a rule, the sections were stained with Heidenhain's iron-hæmatoxylin which was followed by erythrosin or orange G. Such a combination stain seems to differentiate the achromatic structures of the cell, particularly the attraction-sphere and the centrosome, as sharply as it does the chromatin. Safranin followed by lichtgrün and Her-

mann's gentian-violet and safranin also proved of value as they differentiate the chromatin very clearly; they cannot, however, be employed to advantage in a study of the cytoplasmic structures of the cell. Material fixed in corrosive-acetic (5 per cent acetic acid) was stained in all cases with Delafield's hæmatoxylin followed by orange G.

THE PRIMARY SPERMATOGONIA.

Testes of very young toads or those of adults killed in May and June contain many of the large, faintly staining cells to which La Valette St. George gave the name spermatogonia. In the testis of the adult these cells lie, almost invariably, close against the follicle membrane; they are rounded in outline and contain a very large polymorphic nucleus (Fig. 1). A nucleus of this type seems to be characteristic of the primary spermatogonia of amphibians, as it has been found in these cells by most of the workers on amphibian spermatogenesis. A number of small cells, similar in appearance to the cells of the follicle membrane, are always to be found flattened against the primary spermatogonia (Fig. 1, *F. C.*). La Valette St. George (52) was the first to describe such cells in the testes of amphibians; and more recently they have been found by Meves (36) in Salamandra, by Janssens (23) in Triton, and by Kingsbury (29) in *Desmognathus*. It seems very probable that the cells surrounding the primary spermatogonia have the same origin as those on the follicle membrane, and that they are concerned in some way with the formation of the cyst walls which appear as soon as the primary spermatogonia have divided into daughter-cells. The follicle cells are much less noticeable after the secondary spermatogonia are formed; they are crowded in the spaces between adjacent cysts and no longer surround the separate spermatogonia.

The resting nucleus of a primary spermatogonium contains an irregular linin meshwork on which are distributed minute, faintly staining granules of chromatin. A number of rounded nucleoli of various sizes are also scattered throughout the nucleus, being held, apparently, in the meshes of the nuclear reticulum. When material is examined that has been fixed in Flemming's solution and stained with any of the combination stains used, all of the nucleoli invariably take the chromatin stain. If, however, testes are fixed in corrosive-acetic and stained with Delafield's hæmatoxylin and orange G, some few of the nucleoli will stain with the orange, thus showing that they are plasmosomes; the greater number of the nucleoli, however, take the hæmatoxylin and are, therefore, to be considered as chromatin-nucleoli. Janssens also has

found two kinds of nucleoli in the primary spermatogonia of Triton; but I have not been able to confirm his statement that the plasmosomes pass out of the nucleus and serve as nourishment for the cytoplasm. In *Bufo* the plasmosomes are always found in the nucleus until the early prophase of mitosis, when they completely disappear, as do also the chromatin-nucleoli; the latter are doubtless used in the formation of the chromosomes.

In the resting stages of the primary spermatogonium, the centrosome is a minute, deeply staining, spherical body, lying in the cytoplasm near the nucleus and usually in one of the numerous indentations of the nuclear membrane (Fig. 1, *C*). The centrosome at this time is always inclosed in a rounded, granular attraction-sphere that is very similar to the one found by Rawitz (48) in the resting spermatogonia of *Salamandra*.

In addition to the centrosome and its attraction-sphere, there is present in the cytoplasm of the primary spermatogonium a round, or slightly oval, apparently homogeneous body that is considerably larger than the centrosome near which it is generally found (Fig. 1, *A*). Usually, though not invariably, this body is surrounded by a clear area which sharply marks it off from the cytoplasm. It is probable that this structure is to be identified with the "chromatoid Nebenkörper" found by Benda (1) in his study of mammalian spermatogenesis. Benda states that the chromatoid Nebenkörper has nothing to do with the centrosome or with the attraction-sphere, and that it is probably chromatin that has been ejected from the nucleus. He finds that this body disappears in the spermatid and he traces it back to the spermatocyte, but ventures no conjecture as to its probable function. In size and shape this body in the spermatogonia of *Bufo* greatly resembles the smaller chromatin-nucleoli; but in corrosive-acetic preparations stained with Delafield's hæmatoxylin and orange G, it invariably takes the plasma stain and appears as a condensed portion of the cytoplasm. I have not been able to determine the origin of this body which is present in the primary spermatogonia of the young toad long before metamorphosis; but, judging from its staining reactions, it is certainly not chromatin. As will be shown later, this body is undoubtedly concerned in the formation of the acrosome of the spermatozoön, and therefore I suggest for it the name acroblast as somewhat more appropriate than "chromatoid Nebenkörper."

When the spermatogonium is in the early prophase of mitosis, and before the nuclear membrane has broken down, the acroblast becomes constricted through the middle as shown in Fig. 22, *A*, and then divides. The

two acroblasts thus formed separate, and after mitosis is completed one of them is to be found in each of the daughter-cells. An equatorial section of the spindle of a primary spermatogonium during metakinesis occasionally shows the two acroblasts lying together in the cytoplasm near the chromosomes (Fig. 4, A); usually, however, the acroblasts separate before this time and are not to be seen in such a section of the cell. Not infrequently the acroblast can be found on the spindle among the chromosomes (Fig. 9, A). In such cases, if the preparation has been stained with iron-haematoxylin, the acroblast appears as a very small, round chromosome, and it might readily be mistaken for an "accessory" chromosome if its previous history had not been ascertained. The acroblast divides in each of the spermatogonial mitoses and also in both of the maturation divisions, so that one of these bodies is to be found in the cytoplasm of every spermatid. In the spermatid the acroblast undergoes a final division and one part migrates to the anterior end of the spermatid to form the acrosome; the other part remains in the posterior region of the spermatid and eventually disappears.

According to the researches of La Valette St. George (53), Meves (35), Benda (2), and McGregor (34), the primary spermatogonia in the amphibian testes divide amitotically and the descendants of these cells become functional spermatozoa. In *Salamandra*, according to Meves and Benda, this division takes place by means of the constrictive power of a ring-shaped centrosome; in *Amphiuma*, McGregor finds that the nucleus is divided by a simple cleft into two nearly equal parts. Although I have carefully searched through many sections of testes containing large numbers of primary spermatogonia, I have never been able to discover a single cell which I could be sure was dividing amitotically. In the large spermatogonia the nucleus is often found to be much more irregular in outline than that shown in Fig. 1, and not infrequently it appears to be composed of two or more large lobes which are connected only by a very narrow bridge of nuclear substance. In these cases, however, the outline of the cell is invariably rounded, and I have never found any indication of a division of the cytoplasm. If amitotic division of the primary spermatogonia occurs in the testes of *Bufo*, it is exceedingly rare and probably, as suggested by vom Rath (46), the cells dividing in this manner are in the process of dégeneration and never develop into mature spermatozoa.

In every case in which I have observed the division of the primary spermatogonia the process has been taking place karyokinetically. In the early prophase of mitosis all of the nucleoli disappear and the

chromatin, which now stains very intensely, forms into an apparently continuous spireme which breaks into segments before the nuclear membrane disintegrates. There are twenty-four of these chromatin segments, this being the number that is characteristic of the somatic cells of the species. At first these segments are scattered throughout the nucleus, and the marked variation in their size can readily be seen. Figs. 2 and 3 show the total number of chromatin segments in a primary spermatogonium during the early prophase of mitosis; four of the segments are noticeably larger and longer than the rest; four others are very small; and the remaining sixteen segments are intermediate in size between these two extremes. One can readily arrange the chromosomes in twelve groups in which the two members of a group lie near each other and are approximately of the same size. Thus the "pairing" of homologous chromosomes in preparation for the maturation divisions already exists in the original parent cells, as Montgomery (39) has stated is the case in *Desmognathus* and *Plethodon*. A difference in the size of the chromosomes is found in the division stages of all generations of the secondary spermatogonia (Figs. 7-9), and also in both of the spermatocyte divisions (Figs. 43-45, 48-51, 56-57). This furnishes additional evidence in favor of the view advocated by Boveri, Montgomery, Sutton, and others that the chromosomes retain their individuality throughout all stages in the development of the germ-cells. After the nuclear membrane breaks down, the chromosomes condense into broad, V-shaped loops. Sections of the spindle during metakinesis (Figs. 4-5) show a marked difference in the size of the chromosomes; but at this time the chromosomes are so crowded together that it is impossible to determine whether they are arranged on the spindle fibres in any definite way.

About the time that the chromatin spireme breaks into segments preparatory to the division of the cell, the centrosome divides (Fig. 3) and a spindle forms, evidently of extranuclear material. The spindle fibres converge sharply at the poles where the minute centrosomes are clearly seen (Fig. 5). The attraction-sphere, which always surrounds the centrosome during the resting stages of the cell, disappears in the early prophase of mitosis, as it does in the spermatogonia of *Salamandra* (Meves, Rawitz), and of *Amphiuma* (McGregor). It is not again visible until the resting stages of the daughter-cells, as the centrosomes at the poles of the division spindle are totally devoid of any radiation.

THE SECONDARY SPERMATOGONIA.

The secondary spermatogonia are much smaller than the parent cells, and, owing to compression, they are more or less polygonal in outline. The earlier generations of these cells have a conical shaped body with the apex of the cone turned towards the centre of the cyst in which they are contained. As the cells increase in number they are closely crowded together and the cell outlines become constantly more angular and irregular (Figs. 6-9). In the earlier generations of these cells the nucleus is polymorphic in form (Fig. 6), but in the later generations it is usually round or oval. In the resting cell the chromatin is again distributed on a linin meshwork in the form of small granules. These granules stain somewhat more deeply than during the earlier period and their distribution is not so regular (Fig. 6), as in many places groups of them become massed together so that the nuclear reticulum appears jagged and uneven. There are rarely more than five nucleoli in the nucleus of a secondary spermatogonium; of these one or two are plasmosomes, the others karyosomes.

I have never found the secondary spermatogonia dividing amitotically as they do occasionally in *Salamandra* according to Meves. The pro-phases of mitosis in these cells are essentially like those in the primary spermatogonia. All of the nucleoli disappear and the chromatin forms into a thick, apparently continuous spireme which breaks into twenty-four segments of different lengths (Fig. 7). The chromatin segments soon condense into V-shaped chromosomes which are somewhat smaller than those found in the primary spermatogonia.

The spindle in the secondary spermatogonia (Figs. 8, 9) is shorter and somewhat broader than that found in the parent cells (Fig. 5). It also has no radiation around the centrosomes at the spindle poles. The centrosome, attraction-sphere and the acroblast appear in the resting stages of the secondary spermatogonia as they do in the primary spermatogonia.

THE SPERMATOCYTES.

The last division of the secondary spermatogonia gives rise to a new generation of cells, the primary spermatocytes, in which occurs the interesting, yet perplexing, series of changes that brings about a reduction in the number of the chromosomes preparatory to the maturation mitoses.

The resting nucleus of the young spermatocyte usually occupies an eccentric position in the cell. It is invariably round or oval with a smooth contour (Figs. 10, 11) and never has the irregular outline com-

monly found in the cells of the preceding generations. In the very young spermatocyte the chromosomes are still distinct, and they appear as very short, thick rods which are connected by fine linin fibres (Fig. 10). In a slightly older cell, (Fig. 11), the chromosomes are found to be irregular in shape while the linin fibres connecting them are somewhat coarser and stain more intensely than in the younger cell. As the spermatocyte increases in size, the chromosomes gradually disappear, their substance going to form the nuclear reticulum which is constantly growing in amount (Figs. 12-14). At the stage of Fig. 13, minute granules of chromatin are first found scattered along the linin threads. As the substance of the chromosomes becomes distributed throughout the nucleus, the reticulum appears to be composed of a single series of deeply staining, rounded granules which lie so close together that it is almost impossible to make out the linin threads (Fig. 14).

At the end of the growth period of the primary spermatocyte (Fig. 15), the nucleus has increased enormously in volume, and it is very large in proportion to the size of the cell. At this time the chromatin is in the form of a granular, much convoluted spireme which is apparently continuous. The spireme always appears perfectly homogeneous and never shows any indications of a longitudinal splitting.

In the nucleus of the spermatocyte shown in Fig. 11, many of the chromosomes are found to be connected by two parallel linin threads. Such a pairing of the connecting fibres is much more noticeable in the later stages shown in Figs. 12-14. Judging from these figures alone, one might be inclined to think that each chromosome becomes split longitudinally during the formation of the nuclear reticulum in the young spermatocyte, and that the chromatin substance is later distributed along the linin fibres so that the paired threads found at the stages of Figs. 13 14, 18, 19, represent sister portions of longitudinally split chromosomes. This theory might indeed be plausible were the threads of the spireme invariably arranged in pairs at these stages in the development of the spermatocytes; but such is not the case. Sometimes, as shown at the top of Fig. 12, two chromosomes are connected by three linin threads and very often only one fibre connects two chromosomes. The pairing of the chromatin threads is most pronounced at the stage of Fig. 14, and it is rarely found at all at the end of the growth stage when the nucleus contains an apparently continuous spireme (Fig. 15). I do not, therefore, ascribe any special meaning to the pairing of the chromatin threads in the growth stages of the spermatocytes. The spireme is so long and so much convoluted at this time that it would

be very remarkable if some of the threads did not lie parallel for a longer or a shorter distance.

During the growth stages and until the formation of the first maturation spindle the centrosome is always to be found in the portion of the cell containing the greatest amount of cytoplasm (Fig. 15). As in the resting spermatogonia the centrosome is surrounded by a granular attraction-sphere which always appears homogeneous. The acroblast can be found in all of the resting stages of the spermatocytes (Fig. 14), lying usually near the centrosome (Fig. 15). It has the same appearance as in the earlier stages.

Soon after the primary spermatocyte has reached its maximum size, there begins a gradual condensation of the nuclear contents which can readily be followed from its beginning, shown in Fig. 16, to the stage shown in Fig. 22 where the entire contents of the nucleus forms a rounded mass in which it is not possible to make out any details of structure or to determine any of the changes that are taking place. McClung (33) has suggested that the term "synizesis" be applied to the "unilateral or central contraction of the chromatin in the nucleus during the prophase of the first spermatocyte." I shall, therefore, use this term with reference to the stage shown in Fig. 22, in order to avoid the confusion that has resulted from the misuse of the word "synapsis."

The first evidence of the beginning of synizesis is the appearance, near the centre of the nucleus, of a deeply staining mass of tangled chromatin threads from which long loops of the spireme go out in all directions (Fig. 16). At a slightly later stage (Fig. 17), this central mass appears much larger and there are correspondingly fewer loops of the original spireme to be seen. During these early stages in the process of condensation there is frequently found an apparent pairing of the chromatin loops as shown in Figs. 18 and 19; but, as in the growth stages of the spermatocytes, this pairing of the chromatin loops is by no means a constant phenomenon and I cannot but regard it as purely accidental. At the stage of Fig. 20, the greater part of the contents of the nucleus has become condensed into an apparently homogeneous mass from which only a few short filaments of chromatin project. These filaments are very much finer than those of the original spireme and most of them lie parallel with their free ends invariably pointed to that part of the cell in which the centrosome lies. Such a condition of the nucleus as that shown in Fig. 20, is met with so frequently that it is evidently a well defined stage in which there is a definite orientation of the nuclear contents. The gradual condensation of the contents of the

nucleus continues until all of the chromatin is collected in an oval or spherical mass lying usually close to the nuclear membrane with which it is connected by very fine fibres (Figs. 21, 22). In preparations stained with iron-haematoxylin in the usual manner this mass of chromatin appears perfectly black and apparently homogeneous (Fig. 22); if, however, the greater part of the stain is extracted from the sections, this structure is found to be composed of a tangled mass of very fine, granular filaments among which are a number of rounded, deeply staining nucleoli (Fig. 23). These nucleoli, judging from their staining reactions, are not plasmosomes, but more condensed portions of the chromatin substance which retain the haematoxylin with great tenacity.

It can readily be demonstrated that this condensation of the nuclear contents is an actual constructive stage in the normal course of the development of the spermatocytes of *Bufo* and is not "the expression of a running out in the spermatogonium stock and represents a tendency towards degeneration," as claimed by Kingsbury (29) for *Desmognathus*. In the testes of adult toads killed at any time during the summer months, large numbers of spermatocytes can be found showing every step in this process from the stage shown in Fig. 16 to that of Fig. 22, no matter what method of fixation or of staining has been employed. In the testes of toads killed in September and October, the maturation divisions are rarely found, and most of the cysts are filled with spermatids and maturing spermatozoa. If the spermatocytes in which a condensation of the nuclear contents occurs during the summer months are in a state of degeneration, one would expect to find the testes of toads killed in the autumn filled with a large number of cells in the process of disintegration; but such is not the case. It seems quite improbable that large numbers of cells could disintegrate and the debris be disposed of so quickly that it is impossible to obtain any late stages in the process.

I have never found a condensation of the chromatin in the spermatogonia as Kingsbury has described for *Desmognathus*, and I am unable to confirm his statement that "contraction figures do not occur constantly in spermatocytes." In *Bufo* the synzesis stage is most frequently met with in the testes of toads killed in the early summer months simply because at this time the testes contain relatively more cells in this particular stage of development. Contraction figures are, however, to be found in practically any section of the testis, no matter what time of year the toad is killed, and they are found in relatively the same abundance in the testes of young toads as they are in the adult.

From a study of the spermatogenesis of *Batrachoseps attenuatus* Janssens (24) concludes that a condensation of the nuclear contents occurs only when material is badly preserved, and he states that cells of this type are commonly met with in the central mass of tissue where the reagents have not penetrated sufficiently well. Such an explanation will not account for the presence of synizesis in the spermatocytes of *Bufo*. Cells in this stage of development are found as frequently in the cysts at the periphery of the testis as in those at the centre, and not infrequently a cyst filled with spermatocytes in the synizesis stage lies adjacent to a cyst containing dividing cells in which the chromosomes, centrosomes, and achromatic structures are remarkably clear and well preserved. I do not think it possible that a method of fixation that would cause the nuclear contents of one set of cells to become as contracted as that shown in Fig. 22, would not also distort the contents of the other cells of the surrounding tissue to a greater or a less degree.

In none of the amphibians that have so far been studied is the condensation of the chromatin in the primary spermatocytes as complete as in the case of *Bufo*. Janssens (23) states that in the resting stages of the spermatocytes of *Triton* all of the substance of the nucleus is collected in a mass from which numerous filaments extend out to various parts of the nuclear membrane. The synizesis stage in *Triton* is, according to Janssens, about like the stage of condensation in *Bufo* shown in Fig. 20. In *Salamandra*, *Amphiuma*, *Batrachoseps*, and *Plethodon*, according to the investigators who have worked on these amphibians, there is no stage in the development of the spermatocytes in which a condensation of the nuclear contents occurs. The spireme formed after the last spermatogonial division splits longitudinally at a stage about like that of my Fig. 15, and it subsequently breaks into the reduced number of chromosomes. If a condensation of the nuclear contents of the spermatocytes is not characteristic of these cells in all amphibians, it certainly is not confined to *Bufo* and *Triton* as I have already found it in several species of *Rana* and in two of the *Urodela*.

It is obviously impossible to determine what changes are taking place in the chromatin during the time that the substance of the nucleus is massed together in the synizesis stage, as no definite structures, other than those shown in Fig. 23, can be made out even in the most favorable preparations. Judging from the results that have been obtained from a study of the spermatocytes in other species in which a condensation of the chromatin does not occur, it is probable that during synizesis the chromosomes become united end to end in a spireme which later seg-

ments into the reduced number of chromosomes. The "synapsis" stage, to use the term introduced by Moore (41) to designate the stage in which the pseudo-reduction in the number of chromosomes occurs, is in *Bufo* co-incident with the synzesis stage as it is in Triton, elasmobranchs, and various other forms.

A section through a spermatocyte as the cell is emerging from the synzesis stage is shown in Fig. 24. Against one side of the nucleus is a dense irregular mass of chromatin from which extend the loops of an apparently continuous spireme. This spireme differs from that found in the spermatocytes at the end of the growth period, in that it is shorter, thicker, and instead of being granular is very "mossy" in appearance. A slightly later stage in which the mass of chromatin against the nuclear membrane has still further decreased is shown in Fig. 25. At the end of the synzesis stage the nucleus contains a deeply staining, apparently continuous spireme (Fig. 26) which has a much smoother outline than the spiremes shown in Figs. 24 and 25. I have never found any indications of a longitudinal splitting of the spireme that is so characteristic of the post-synapsis stages in the spermatocytes of the Urodela.

Soon after the stage shown in Fig. 26, the spireme shortens somewhat and, consequently, becomes considerably thicker than before (Fig. 27). Subsequently certain portions of the spireme stain much more lightly than the rest, these lighter staining regions marking the location of the transverse divisions by which the spireme is later broken into the reduced number of chromatin segments (Fig. 28). The next step in the process of separating the chromosomes is seen in Fig. 29, where the spireme is found to be constricted at various places. In many cases, before this first constriction of the spireme is completed, a second constriction appears through the middle of each segment so that the chromosomes that are finally produced from the spireme have the shape of dumbbells (Fig. 30). It is very probable that the constriction in each segment marks the line of union of the two chromosomes that were united end to end in synapsis; and, as will be shown later, this constriction also marks the location and the direction of the division of the chromosomes in the first maturation mitosis of the spermatocytes. When the chromosomes have the shape of dumbbells at the stage of Fig. 30, they undergo no further changes in form until the anaphase of the first maturation mitosis. There is no indication of the second division in this type of chromosome until the late anaphase when the chromosomes are again constricted into dumbbells (Fig. 51). If one traces the various steps in the development of the chromosomes from the stage represented by Fig. 27 until

the time that mitosis is completed by the division of the chromosomes through the middle of the dumbbells (Figs. 41, 42, 44, 45, 48), there seems no possibility of avoiding the conclusion that the first maturation mitosis in the spermatocytes of *Bufo* is a transverse or reduction division in the Weismannian sense, separating chromosomes that were united end to end in synapsis.

In some of the spermatocytes the spireme formed after the synzesis stage does not produce dumbbell-shaped chromosomes, but breaks into rectangular blocks (Fig. 35). These rectangular shaped chromosomes also maintain their definite shape up to the metaphase, and are never divided either for the first or for the second maturation mitosis before this time. Owing to this persistence in the shape of the chromosomes there can be no doubt but that here too the first division is a reduction division; as in the anaphase the chromosomes are nearly square and not long and narrow as would be the case if they were divided longitudinally (Fig. 52).

An examination of Figs. 30-36 will show that before the separation of the chromosomes there is always a considerable variation in the size of the segments in the same nucleus. This difference in the size of the chromosomes is maintained throughout all of the subsequent stages of mitosis and shows very clearly in the equatorial plate of the first maturation spindle shown in Fig. 43. All twelve of the chromosomes are present in this case and two of them are considerably larger than the others. In the early prophase of the division of the primary spermatogonium shown in Figs. 2 and 3, there are four of the chromosomes that are noticeably larger than the rest. It seems very probable that these chromosomes maintain their individuality throughout all of the spermatogonial divisions and are united two and two in synapsis to reappear in the prophase of the first maturation mitosis as the two bivalent chromosomes that are readily distinguished from the others by their size. If this is true for four of the spermatogonial chromosomes it is, of course, true for all. Thus the twelve chromosomes shown in Fig. 43 are bivalent structures formed by an end to end union of homologous chromosomes which, as Montgomery (39) has shown, are to be traced back through the divisions of the secondary spermatogonia to the primary spermatogonia.

The difference in the size of the chromosomes on the first maturation spindle is strikingly illustrated in Fig. 48 where one of the largest of the chromosomes lies next to one of the smallest, the mass of the former being more than twice that of the latter. In the metaphase and early anaphase of the second maturation division (Figs. 56, 57), one can also

perceive a difference in the size of the chromosomes, although this difference is not so marked as in the first mitosis because of the smaller size of the chromosomes. Many investigators have already noticed a variation in the size of the chromosomes in the maturation divisions of the germ-cells. Were the reasons for this phenomenon known, they would doubtless aid materially in solving many of the vexing problems of heredity.

In a great majority of the spermatocytes the post-synapsis stages of development do not follow the well marked types described above, but are irregular and somewhat difficult of analysis. In many cases the spireme, instead of breaking into dumbbell or rectangular-shaped segments, forms the ring-shaped chromosomes that are so characteristic of the prophases of the first maturation mitosis in the spermatocytes of other animals. In most of the cases that have been described, the ring-shaped chromosome is produced by the longitudinal splitting of a chromatin segment which opens through the middle region and remains united at the ends, a typical case being that of *Grylotalpa* as described by vom Rath (45). In *Bufo* the method of ring formation is somewhat peculiar. The spireme formed after the synzesis stage constricts into oval segments (Figs. 32-34), and usually, before the constriction is completed, the segments open through the middle, thus forming typical ring-shaped chromosomes which may remain connected for some time (Figs. 31, 32, 33, 36). In *Bufo*, therefore, the opening in the ring is not the space between univalent chromosomes, as maintained by Montgomery (38) for *Desmognathus* and *Plethodon*, but represents the longitudinal splitting of a bivalent chromosome that is completed in the second maturation mitosis. The first maturation mitosis, which is always seen at this stage in the dumbbell-shaped chromosomes (Figs. 30, 37, 38), is not, as a rule, visible in the ring-shaped chromosomes until they have entirely separated and condensed into tetrad groups, as do the ring-shaped chromosomes in *Grylotalpa* (Fig. 39). Before the first maturation spindle is formed the ring-shaped chromosomes become scattered throughout the nucleus (Fig. 40). They are at this time usually round or oval in form, but occasionally they are distinctly diamond shape (Figs. 32, 40). In only two cases have I ever seen any indication of a division of the ring-shaped chromosomes preparatory to the first maturation mitosis. One of these cases is shown in Fig. 32, where, at the four corners of the diamond, dark lines divide the chromosome into four parts, thus plainly indicating that the chromosome has already divided for both of the maturation mitoses.

It is apparently a matter of little, if of any, importance which of

these various forms the chromosomes assume in the prophases of the first maturation mitosis, as frequently in the same nucleus some of the chromosomes have the form of dumbbells while others are in the shape of rings (Fig. 38). Nuclei containing chromosomes of different shapes are perhaps the most valuable for a study of this period in the development of the spermatocytes, as they show very clearly how the different types are related to each other and to the original spireme. Fig. 38 shows a section of a spermatocyte in which the chromosomes are of mixed types. Three dumbbell-shaped chromosomes, in which the direction of the first maturation mitosis is indicated by the **constriction** in the middle of the chromosomes, are connected with a ring-shaped chromosome which shows only the second division. As these chromosomes are still united end to end by linin fibres there can be no question of the relation of these two types of chromosomes to each other and to the original spireme.

A section of another spermatocyte of the same character is shown in Fig. 39. In this case not only are the chromosomes of different types but they are also in slightly different stages of development. As the chromosomes are entirely separated it is not possible to determine their relation to the original spireme. This cell is unique in that it shows in the same nucleus chromosomes in which the first or the transverse division has already begun (dumbbells), chromosomes in which the second or the longitudinal division only is apparent (rings), and one chromosome which has already divided for both of the maturation mitoses (tetrad).

Although ring-shaped chromosomes are found abundantly in the prophases of the first maturation mitosis, I have never found one of them in the equatorial plate of the spindle where they are always to be found in *Salamandra*, *Amphiuma*, and others of the Urodela. In a study of the spermatogenesis of *Pedicellina americana*, Dublin (11) found that the chromosomes have the form of rings in the prophase of the first maturation mitosis. When the spindle is formed these chromosomes elongate considerably, but still retain their ring-shaped character during the early metaphase. In the late metaphase the chromosomes lose their former shape and condense into elongated rods which divide transversely, the division being reductional. Thus *Pedicellina* seems to bridge over the gap between such forms as *Salamandra* and *Amphiuma* in which the ring-shaped chromosomes persist throughout the entire metaphase of the first maturation mitosis, the ensuing division being of the hetero-

typic type, and forms like *Gryllotalpa* and *Bufo* in which rings give place to tetrad groups before the metaphase and division is reductional.

Where tetrads are formed in the prophase of the first maturation mitosis it is, of course, not possible to determine whether the first or the second division is reductional. As the first maturation division of the chromosomes that have the shape of dumbbells and also of those that take the form of rectangular blocks is transverse, it seems safe to assume that the tetrads are so placed on the spindle that univalent chromosomes are separated in the first mitosis.

The formation of the first maturation spindle evidently takes place very quickly, as I have been able to find only a comparatively few stages in its development. Soon after the synzesis stage the centrosome divides and the attraction-sphere disappears as it does before the formation of the spindle in the spermatogonial mitoses. As the centrosomes move apart, each becomes the centre of a very small aster formed, presumably, of the substance of the attraction-sphere (Figs. 36, 41). As a rule, the centrosomes lie at this time very close to the nuclear membrane which is somewhat irregular in outline. After the disappearance of the nuclear membrane, a small spindle is found with the centrosomes, surrounded by small asters, at the spindle poles (Fig. 42). The spindle grows rapidly, probably at the expense of nuclear material, and when fully formed in very large proportion to the size of the cell (Figs. 44-53). After the stage of Fig. 42 every trace of the polar radiation disappears and the centrosomes are totally devoid of any radiation in all subsequent stages.

During, or soon after the synzesis stage, the acroblast becomes dumb-bell shape (Fig. 22, *A*) and then constricts into two rounded bodies (Fig. 26). This division takes place before the centrosome divides, for, as shown in Fig. 26, one can occasionally find a section of a cell containing the centrosome surrounded by its granular attraction-sphere and two acroblasts. During the formation of the spindle the acroblasts usually separate and in the metaphase are to be found some distance apart (Fig. 48). Occasionally, as in the spermatogonial divisions, the acroblasts lie close together in the equatorial region of the spindle and appear as very small chromosomes (Fig. 45, *A*). During the anaphase the acroblasts have apparently no definite position in the cell as they may be found on the spindle, near to it, or close against the cell wall (Figs. 50, 53). They are, however, at this time always on opposite sides of the equator of the spindle, and when cell division takes place one acroblast goes to each of the daughter-cells.

During the late anaphase of the first maturation mitosis, the chromosomes become greatly crowded together and appear to fuse into an amorphous mass in which the outlines of the separate chromosomes are completely lost (Fig. 53). Whether there is a true fusion of the chromosomes at this time I have been unable to determine. Kingsbury has found a similar massing of the chromosomes at the poles of the first maturation spindle in the spermatocytes of *Desmognathus*, and he believes that the chromosomes do not lose their identity at this time although they become inclosed in a nuclear membrane. Division does not take place simultaneously in all of the spermatocytes of a cyst; sometimes only a single cell will be found dividing while all of the other cells of the cyst are in the early or late pro phases of mitosis.

As in *Salamandra* and *Triton*, there is no resting nucleus formed between the two maturation divisions. Sometimes the centrosomes divide for the second mitosis during the early anaphase of the first mitosis (Fig. 50), but as a usual thing division does not take place until much later. As in the pro phases of the former divisions, each centrosome is, for a time, the centre of a small aster (Fig. 54). As the spindle for the second mitosis forms, the asters disappear and in the fully formed spindle the fibres converge sharply to minute centrosomes which show no traces of a radiation.

During the formation of the second maturation spindle, the chromosomes remain massed together, as shown in Fig. 54, and the changes taking place in them cannot be determined. In rare instances this amorphous mass of chromatin may be found at the equator of the fully formed spindle (Fig. 55), but as a rule the chromosomes are separated at this time. Part of an equatorial plate of the second maturation spindle is shown in Fig. 56. Nine dumbbell-shaped chromosomes are seen which, as one might expect, are very much smaller than those found on the first spindle. The fact that at this stage also the chromosomes are of different sizes seems to indicate that the chromosomes maintain their individuality during the period between the two maturation divisions when they are apparently fused into an irregular mass. The second division must take place much more quickly than the first, as I have not been able to find more than two or three dividing cells in sections of the testis containing hundreds of cells in earlier or slightly later stages of development. In all of the spindles that I have seen, the chromosomes were invariably in the form of dumbbells in the metaphase (Fig. 56). In the anaphase, each dumbbell is separated into two nearly spherical portions (Fig. 57) which are inclosed in a membrane

before the division of the cell is completed. The second mitosis is longitudinal, completing the division that is begun in some of the chromosomes at the stage of Fig. 31, and in others not until the late anaphase of the first mitosis (Fig. 51).

THE SPERMATIDS AND SPERMATOZOA.

The nucleus of the young spermatid occupies a very eccentric position, lying in the portion of the cell that is to become the head of the spermatozoon (Fig. 58). At first the chromatin is distributed throughout the nucleus in the form of angular shaped blocks connected by fine linin threads (Fig. 58); later it is spread out in an irregular reticulum (Figs. 59-65). During the development of the spermatid the nucleus loses its rounded shape and becomes greatly elongated, finally appearing in the mature spermatozoon as a cylindrical, perfectly homogeneous body that has great affinity for all chromatin stains.

The centrosome is always found in the part of the cell containing the greatest amount of cytoplasm. It is not surrounded by a granular attraction-sphere as in the resting stages of the preceding generations, but is inclosed in a clear, round or oval vesicle which is sharply marked off from the surrounding cytoplasm (Figs. 58, 59, etc.). Whether the substance of this vesicle is derived from the attraction-sphere of the spermatocytes I have not been able to determine. The attraction-sphere disappears in the prophase of the first maturation mitosis and I have found no traces of it in later stages, unless, indeed, the radiation found for a short time around the centrosomes as the second maturation spindle is forming is derived from the substance of the attraction-sphere.

In the early stages of the development of the spermatids, the vesicle inclosing the centrosome may lie freely in the cytoplasm (Fig. 60), or it may be in contact with the nuclear membrane (Fig. 58); in the latter case it marks the region of the nucleus that is to become the posterior end of the sperm-head, as the vesicle itself is the anlage of the middle-piece of the spermatozoon. The centrosome divides soon after the stage shown in Fig. 58, and in favorable preparations one can see that the two centrosomes are connected by a very fine, thread-like fibre (Figs. 59, 60). As the centrosomes move apart, the vesicle inclosing them elongates and later it becomes much flattened where it presses against the nuclear membrane (Figs. 61, 62, etc.). The middle-piece anlage appears perfectly transparent during all stages of its development and shows not the slightest affinity for either hæmatoxylin or safranin stain. In the mature spermatozoon, the middle-piece has the same diameter as

the sperm-head and forms a well marked division between the head and the tail (Fig. 71, *M. P.*).

One centrosome, which for convenience I shall call the "inner" centrosome, moves to the edge of the vesicle in contact with the nucleus and for a time appears as a small rounded projection from the middle of the posterior nuclear wall (Figs. 62, 63, 65). Later this centrosome becomes imbedded in the deeply staining substance of the sperm-head; whether it aids in the formation of the middle-piece, as Meves finds is the case in the spermatids of *Salamandra*, I have been unable to determine. The "outer" centrosome, still keeping its connection with the inner centrosome, moves to the posterior end of the vesicle and from it a fibre grows out that later forms the axial-filament of the tail (Figs. 61-63, etc.). In the spermatids of *Salamandra* and of *Amphiuma*, the outer centrosome becomes ring-shaped and later divides into two parts: one part remains at the posterior end of the middle-piece; the other part migrates down the axial-filament to the beginning of the end-piece of the tail. Owing to the fact that the spermatids in the testis of *Bufo* are very much smaller than those of the *Urodela* and that the posterior region of the cell shows little affinity for either plasma or chromatin stains, it is very difficult to follow the history of the outer centrosome. As far as I have been able to determine, this centrosome never divides, but remains at the posterior end of the middle-piece where it soon becomes disc shaped (Figs. 66-68), and later flattens considerably (Figs. 69-70). In favorable preparations of the mature spermatozoön, this centrosome appears as a more deeply staining mid-portion of the posterior border of the middle-piece; in many cases it cannot be found at all.

In the young spermatid, the acroblast appears round or oval and homogeneous as in the earlier stages. It is always found in the posterior region of the cell, usually lying some distance from the centrosome from which it is readily distinguished on account of its larger size (Fig. 58). During an early period in the development of the spermatid, the acroblast again divides, and occasionally a cell is found containing both of the centrosomes and two acroblasts which are considerably smaller than those found in the spermatocytes (Fig. 63). One of the acroblasts remains in the posterior part of the cell (Figs. 66, 68), and as the tail forms it stains less intensely than before and subsequently disappears. The other acroblast moves gradually towards the anterior end of the spermatid, as shown in Figs. 65-67. Transverse sections of the spermatids will occasionally show this acroblast lying just outside of the

nucleus surrounded, as in the earlier stages, by a clear area (Fig. 64). The acroblast finally reaches the anterior end of the cell (Fig. 67), and then comes in contact with the nuclear membrane (Fig. 68). Subsequently the acroblast flattens against the apex of the nucleus (Fig. 69) and seems to fuse with it, forming a deeply staining, cap-like body (Fig. 70, 71, *Ac.*). The cytoplasm in the anterior region of the cell later forms an awl-shaped apical body in front of the acrosome, which stains very faintly with plasma stains (Fig. 71).

During the later growth stages of the spermatids, the cysts inclosing them become disorganized and a cavity appears in the middle of the follicle. This cavity contains a considerable amount of débris formed from the degeneration of the cyst membranes and the follicle cells. Some of this débris is in the form of large and small granules which take the chromatin stain. It is, therefore, somewhat difficult to follow the history of the acroblasts, as frequently a spermatid will be found that appears to contain several rounded, deeply staining granules of about the same size as the acroblast. Fortunately, the acroblast in the spermatid is almost invariably surrounded by a clear area both before and after it has divided (Figs. 58, 61, etc.), and thus it can usually be distinguished from granules of débris that often appear to be a part of the cell. In studying the history of the acroblast I have made use of sections of the testis that were purposely crushed and broken; as by such means the spermatids, which are normally crowded close together, are separated and partially freed from other material in the follicle.

The mature spermatozoön of *Bufo lentiginosus* has already been described and illustrated (King, 26). The head is a long cylindrical structure which is seemingly homogeneous after the usual staining with iron-hæmatoxylin. If, however, the stain is partially extracted, as was done in the preparation from which Fig. 71 was drawn, the head appears grayish in color and there are two deeply staining regions, one at the anterior, the other at the posterior end. It is evident that the parts of the sperm-head that retain the stain with the greatest tenacity mark the location of the two bodies that entered the nucleus at an early period in the development of the spermatid. At the posterior end of the sperm-head lies the inner centrosome which entered the nucleus of the spermatid just before the stage of Fig. 66; and at the anterior end is the acroblast which fused with the nucleus at about the stage of Fig. 69. Both of these structures evidently persist in the mature spermatozoön and retain their great affinity for the iron-hæmatoxylin stain.

The middle-piece of the mature spermatozoön (Fig. 71, *M. P.*) has

the same diameter as the sperm-head, but it is sharply marked off from it and shows not the slightest affinity for chromatin stains. In rare instances, as shown in Fig. 71, a slender fibre extends through the entire middle-piece. This fibre is evidently the connection between the centrosome at the posterior end of the middle-piece and the centrosome imbedded in the end of the sperm-head which has persisted through all stages in the development of the spermatid. As a rule, the connection between the centrosomes is broken after the stage of Fig. 65, and in the older spermatids and in the spermatozoa the middle-piece appears perfectly homogeneous.

The tail of the spermatozoön is very long and it is composed of two filaments which are connected by a thin, transparent, undulating membrane. The axial-filament, which grows out of the outer centrosome, is somewhat thicker than the marginal-filament and it extends some distance beyond the latter to form the end-piece of the tail.

GENERAL DISCUSSION.

As long ago as 1887, Flemming (15) described typical tetrad groups in the testes of *Salamandra*, although he considered these structures to be "anomalies" and not normal stages in the development of the spermatozoa. Later vom Rath (46, 47) maintained that tetrads are normally present in the testes of *Rana* as well as of *Salamandra*, and that one of the maturation divisions is a transverse or reduction division in the Weismannian sense. With but few exceptions, all of the investigators who have recently worked on the spermatogenesis of amphibians have insisted that both of the maturation divisions are equatorial, and that normally tetrad groups are not present in the spermatocytes. This later work on amphibians, and the seemingly exhaustive studies of Brauer (5), Boveri (3), and Hertwig (22) on the germ-cells of *Ascaris*, together with the work of Strasburger (56, 57) and other botanists on the flowering plants, has given strong support to the view that reduction in the germ-cells can be effected either by a double longitudinal division or by one transverse and one longitudinal division, the division of the chromatin substance being the main thing and the manner of its achievement quite secondary. The most recent studies on spermatogenesis, and oogenesis, and experiments made by Boveri, Morgan, and others, have seemed to show that the chromosomes maintain their individuality from one generation to the next. If this is true, then in the maturation mitoses, reduction must in all cases be brought about by one transverse

and one longitudinal division of the chromosomes, as the individuality of the chromosomes is lost if two equational or two transverse divisions occur.

Sebaschnikoff's (5) re-examination of the oögenesis of *Ascaris* has made it doubtful whether, after all, the tetrad groups are here formed by a double longitudinal division; Boveri (4) and Montgomery (40) have more recently brought forward strong arguments in favor of the occurrence of a reduction division in the germ-cells of *Ascaris*; and Strasburger (58), after a re-examination of his material, is now of the opinion that a reduction division also occurs in the higher plants. It remains only to bring the investigations on the germ-cells of the amphibians into line with these later investigations on other forms to establish the rule that there is one reduction and one equational division in the maturation of the germ-cells of all animals and plants so far investigated. There is, apparently, a great diversity in the way in which reduction is accomplished in the various forms.

Most of the investigators who have worked on the spermatogenesis of the Urodela agree that, in the early prophase of the first maturation division, the reduced number of chromosomes appears in the form of U- or V-shaped loops; and they have tacitly assumed, if not expressly stated, that each loop is a bivalent structure being composed of two chromosomes united end to end in synapsis. Later these chromatin loops split longitudinally and the sister-portions of each loop remain united at the ends, opening up through the middle to form ring-shaped chromosomes. In metaphase the rings are said to be placed on the spindle in such a way that the plane of the union of the two halves of the chromosomes lies in the equator of the spindle. The heterotypic division which follows separates sister-portions of the longitudinally split chromosomes and is therefore an equational division. In the anaphase the V-shaped chromosomes again split longitudinally preparatory to the second maturation mitosis which is also an equational division.

Montgomery, who is a firm advocate of the view that the first maturation mitosis must necessarily be reductional, has investigated the prophase of the first maturation division in the spermatocytes of two amphibians, *Plethodon* and *Desmognathus*, and his interpretation of the ring-formation in these forms differs considerably from that given by Meves, McGregor, and Eisen (11). In the early prophase of mitosis, Montgomery also finds the reduced number of chromosomes in the form of loops which he considers to be composed of two chromosomes united end to end in synapsis; each arm of a loop representing one chromo-

some and the angle of the loop indicating the place of their union. At a later stage the free ends of each loop become cemented together forming ring-shaped chromosomes. "Hence in the typical chromosome of the ring form, the space enclosed by the chromosome is the space between two univalent chromosomes and has nothing to do with the longitudinal split." Montgomery asserts that the longitudinal splitting of the chromosomes in preparation for the second maturation mitosis appears in the arms of the loops before the rings are formed, and that it has been overlooked by the other investigators, as it is completely hidden in the metaphase and only reappears in the anaphase as a longitudinal splitting of the daughter chromosomes. Montgomery believes, therefore, that the so-called heterotypic division in the species of amphibians that have so far been investigated is a true reduction division in the Weismannian sense and that the second division only is a longitudinal one. He ventures the prediction that this will be found true for all forms in which the heterotypic division is found to occur.

The interpretation given to the ring-formation by Montgomery has been criticised by Janssens and Dumez (25) who insist that in *Plethodon* both of the maturation divisions are longitudinal. Janssens (24), however, in a more recent paper dealing with the development of the spermatocytes of *Batrachoseps attenuatus*, seems to have changed his former view as he states that "il est extrêmement probable, pour ne pas dire plus, que les 12 anses du bouquet chez le *Batrachoseps* résultent de la soudure deux à deux suivant toute leur longueur des 24 chromosomes des dernières cinèses somatiques." This conjugation of the chromosomes leads later to the formation of dyads "qui seront séparées pendant la première cinèse de maturation ou hétérotypie. Il est donc très probable que les deux spermatocytes II reçoivent chacun 12 chromosomes entiers, c'est-à-dire la moitié des 24 chromosomes des dernières cinèses spermatogoniales." Besides supporting Montgomery's contention that the first maturation division in the spermatocytes of amphibians is a reduction division, this work of Janssens is very valuable in another way, as it is the first work on the spermatogenesis of amphibians that suggests the possibility of a side by side union of the chromosomes during synapsis. I do not see why it is necessary to assume, as have the majority of the investigators on amphibian spermatogenesis, that the chromosomes must always unite end to end in synapsis. Evidence is not lacking that a conjugation of the chromosomes in pairs may take place previous to the first maturation division. Thus Rückert's (49,50) early observations on the oogenesis of *Pristiurus* seems to show a side by side union of the

chromosomes, although Rückert considers that the paired arrangement of the chromosomes may be due to "eine eigentümliche Langsspaltung der Chromosomen im Dyaster der letzten Teilung des Ureies." More conclusive evidence is given by Calkin's (8) work on *Lumbricus*. In this form the spireme first splits longitudinally and then segments into the somatic number of segments, 32. These double segments then conjugate in pairs forming 16 tetrad groups. The first maturation division is a reduction division, separating the pairs of chromosomes that conjugated to form the tetrads. More recently Steven's (55) investigations on the germ-cells of Aphids have shown that there is in these forms undoubtedly a pairing of the chromosomes previous to the first maturation division. If a more extended investigation of the spermatocytes of the Urodela should make it seem probable that Janssen's interpretation of synapsis in *Batrachoseps* can be extended to other species, then univalent chromosomes will be separated in the first mitosis in all such cases, and the maturation divisions in this group of amphibians will readily fall in line with those of all other accurately known forms.

In studying the spermatogenesis of *Bufo* I have been fortunate enough to find practically all stages in the development of the primary spermatocytes from the time that the cell is formed (Fig. 10) until the completion of the first maturation division (Fig. 53); and I have traced, as carefully as possible, the complex changes taking place in the chromatin at this time. In the young spermatocyte shown in Fig. 10, the chromosomes are distinct and connected by fine linin fibres. At this stage the cells are very small and many of the chromosomes are crowded against the nuclear wall so that I have not been able to make out their number satisfactorily. As there are certainly many more than 12 chromosomes in the nucleus at this time, I am very sure that pseudo-reduction has not yet taken place and that the somatic number of chromosomes, (24) is present. As shown in Fig. 10, the chromosomes in many cases appear to be connected end to end, especially is this noticeable in the chromosomes that lie against the nuclear wall. In the later development of the spermatocytes the chromatin substance becomes distributed on the nuclear reticulum and all traces of the individual chromosomes is lost. If, however, an end to end union of the chromosomes is established at or before the stage of Fig. 10, it is safe to assume that this connection is not broken during the later growth stages of the spermatocytes.

I have never found any evidence of the longitudinal splitting of the spireme in the young spermatocytes that has been found at the same

stage of development in the spermatocytes of several of the Urodela. Up to the stage of Fig. 14, the chromatin reticulum in the spermatocytes of *Bufo* is very fine and one can readily see that the chromatin granules are arranged serially. The continuous spireme found at the stage of Fig. 15 is always perfectly homogeneous. Although I have examined large numbers of spermatocytes very carefully with this particular point in mind, I have never been able to find a single cell in which there was the slightest evidence of a longitudinal splitting of the spireme at the stage of Fig. 15, nor during any stages showing the condensation of the nuclear contents. I have already stated that I regard the apparent pairing of the chromatin threads shown in Fig. 13, 14, 18, 19, as purely accidental. In most cases the parallel threads are some distance apart, this would probably not be the case if a longitudinal splitting of the spireme had occurred; again, the threads are never connected by fine fibres, as is usually the cases with sister portions of a longitudinally split chromatin skein; most important of all, during the early stages of the formation of the nuclear reticulum, the threads are always composed of a single series of chromatin granules and they are all of the same thickness whether they are single or in pairs.

Recently several investigators, among whom may be mentioned A. and K. E. Schreiner (54), and von Winiwarter (61), have maintained that the apparent longitudinal splitting of the chromatin loops during or soon after the "bouquet" stage is, in reality, a folding together of the chromatin filaments so that two of them come to lie parallel and thus produce the appearance of a split filament. Synapsis, according to this interpretation, is brought about by a side by side conjugation of the chromosomes and not by an end to end union. A folding together of the parts of the chromatin skein is exactly what occurs in the young spermatocytes of *Bufo* at the stages of Figs. 13, 14, 18, 19; but this arrangement always occurs before the synzesis stage and never after it, and it is found in only a small minority of the spermatocytes. Were this pairing of the chromatin threads interpreted as a conjugation of univalent chromosomes that is to persist throughout the synzesis stage and until the metaphase of the first maturation division, then, as will be shown later, both of the maturation divisions would of necessity be reduction divisions and there would be no way in which the individuality of the chromosomes could be maintained from one generation to the next. I cannot, therefore, believe that the apparent pairing of the chromatin threads in the young spermatocytes of *Bufo* is of any great importance, although I do not question but that it may be a constant and important stage in the development of the germ-cells in other forms.

The condensation stage in the primary spermatocytes of *Bufo* is much more marked than in any of the other amphibians that have so far been investigated, and it is evidently a stage of relatively long duration, judging from the number of cases that are to be found in every section of the testis of adult toads killed during the summer months. The changes occurring in the chromatin during this time cannot, of course, be ascertained; but it is evident, from an examination of iron-haematoxylin preparations that have been considerably destained (Fig. 23), that the chromatin does not become a homogeneous mass. In light of the results obtained from the study of the maturation phenomena in the germ-cells of other forms in which there is no synizesis stage, or one much less marked than in *Bufo*, it would seem as if the final steps in the process of synapsis must take place at this time although I am very much inclined to believe that in *Bufo* this process may have had its beginning in the very young spermatocytes (Figs. 10, 11).

Whatever the changes taking place during synizesis, the chromatin emerges from this stage in the form of a continuous, homogeneous spireme. Although I have examined hundreds of spermatocytes at the stages of Figs. 24-28, I have never found the slightest indication of a longitudinal splitting of the spireme at this time. Such a splitting is first evident at the stage of Figs. 31-33, and then only in cases in which ring-shaped chromosomes are being formed.

If the chromosomes conjugated side by side during synapsis so that the opening in the ring-shaped chromosomes shown in Figs. 32, 33, 36, and 38 represents the space between two univalent chromosomes, as Montgomery has maintained, then the first maturation mitosis, which undoubtedly divides the tetrads formed from the rings in the same way that it does the dumbbell-shaped chromosomes, does not separate univalent chromosomes, but cuts each univalent chromosome in half and is, consequently, a reduction division. On the same assumption, the second division would be in the plane of the union of the univalent chromosomes and would separate the two remaining parts of each chromosome, being also a reduction division. Thus if we consider that the rings in the spermatocytes of *Bufo* are formed of two chromosomes *A* and *B*, which have conjugated side by side in synapsis as shown in diagram I, the first maturation division through the line *X* would cut each chromosome into two parts: *A* would be divided transversely into *a'* and *a''*; while *B* would be divided into *b'* and *b''*. The second division through the line *Y* would separate *a'* from *b'* in the one cell and *a''* from *b''* in the other cell. Both of the divisions being reduction divisions, the in-

dividuality of the chromosomes would not be maintained any more than if a double longitudinal division occurred. Wilcox (59) is, I believe, the only investigator who maintains that both of the maturation divisions are transverse, and his results have not been confirmed by those of other workers on insect spermatogenesis.

If, as seems more probable, synapsis takes place in the spermatocytes of *Bufo* by an end to end union of the chromosomes, the maturation divisions are diagrammatically represented by Diagram II. The first maturation division, through the line *X*, separates the univalent chromosome *A* from the univalent chromosome *B*, and is thus a reduction division. The second division, through the line *Y*, separates *A* into two equal parts *aa*, and *B* into *bb* and is, consequently, an equation

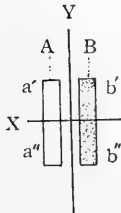


DIAGRAM 1.

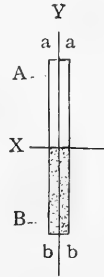


DIAGRAM 2.

division. It seems to me a matter of no great importance as to which mode of union takes place in synapsis provided that, in the subsequent mitoses, one division is reductional and the other longitudinal: the final result is the same in both cases. I agree fully with the conclusion reached by Grégoire (18), Farmer and Moore (12), and Montgomery (40), after an exhaustive résumé of the work done on the maturation phenomena in the germ-cells of animals and plants, that reduction occurs in the first maturation mitosis, the second division being longitudinal.

In none of the forms that have so far been studied is the evidence in favor of a reduction division in the maturation mitoses of the spermatocytes more convincing than in *Bufo*. The mode of formation of the dumbbell-shaped chromosomes in the early prophase of the first maturation mitosis and their later division across the middle of each dumbbell furnishes indisputable evidence that the first division is a reduction division in the Weismannian sense. That tetrads are normal structures in the spermatocytes of *Bufo* seems also indisputable. I have found tetrad groups in large numbers in the testes of adult toads killed at

various times during the summer months and also in the testes of young toads, so that in *Bufo* it is impossible that they can be "anomalies." The results of my work on *Bufo*, therefore, are in full accord with those obtained by vom Rath on *Rana* and *Salamandra* which have been ignored or severely criticised by other investigators of amphibian spermatogenesis.

The evidence brought forward by investigators who have studied the maturation phenomena in the eggs of amphibians has been overwhelmingly in favor of the occurrence of a double longitudinal division of the chromosomes previous to the extrusion of the polar bodies. Carnoy and Lebrun (9), and Lebrun (30, 31) have published a series of memoirs dealing with the formation of the polar bodies in the eggs of various amphibians, and they have emphatically denied the occurrence of a reduction division in the forms that they have studied. In my earlier study of the maturation of the egg of *Bufo lentiginosus* (King, 26) I was inclined to believe that both of the maturation divisions are longitudinal; but a later examination of a much more complete series of preparations of the first polar spindle (King (27)) led me to suggest the probability that the first maturation division is a reduction division. In light of the present study of the spermatogenesis of *Bufo*, and of investigations, not yet completed, on the oogenesis of this amphibian, I am convinced that in the oocytes as well as in the spermatocytes the first maturation division is a reduction division and that the second division only is longitudinal.

Although all investigators agree that the head of the mature spermatozoön is derived from the nucleus of the spermatid, there is no such unanimity of opinion regarding the origin of the other parts of the spermatozoön.

The middle-piece of the amphibian spermatozoön has been described as arising from the chromatin of the nucleus (Bühler, Flemming (16)) from the inner centrosome (Meves); from a part of the idiozome (McGregor) and from a nebenkörper (Hermann (20)). In the very young spermatids of *Bufo* the middle-piece anlage is frequently found some distance from the nucleus (Fig. 60), and there is therefore very little probability that it is a nuclear product. The appearance of the anlage of the middle-piece before the centrosome has divided precludes the possibility that it is derived from the inner centrosome. If, therefore, this structure is other than modified cytoplasm, it is very probably derived from the idiozome. As there is no structure in the spermatid at this time that at all resemble an idiozome, it must be that the entire

idiozome is transformed into the middle-piece and not merely a part of it as in *Amphiuma*. The results of my investigation of the origin of the middle-piece do not agree with those obtained by any other investigator of amphibian spermatogenesis, although they accord with Calkin's account of the origin of the middle-piece in *Lumbricus*.

As far as I am aware, Fick (13) is the only investigator who has found that the middle-piece of the mature spermatozoön of amphibians shows any great capacity for staining either with chromatin or with plasma stains. Fick states that the middle-piece of the spermatozoön of *Axolotl* stains intensely black after the use of iron-hæmatoxylin, whereas in *Bufo* and in other amphibians it remains nearly colorless. This difference in staining reactions suggests that the inner centrosome in the *Axolotl* spermatozoön remains in the middle-piece and does not go into the posterior end of the head as it does in other forms.

The history of the centrosomes in the spermatid of *Bufo* is similar in many ways to that of the centrosomes in the spermatid of *Salamandra* and of *Amphiuma*, yet it differs in several important respects. In *Bufo*, as in the *Urodela* that have been most carefully studied, the axial filament of the tail grows out from one of the centrosomes while the other passes into the posterior end of the head. Whether this latter centrosome takes any part in the formation of the middle-piece, as Meves has stated for *Salamandra*, I have not been able to determine.

The history of the outer centrosome in the spermatids of *Bufo* is not easily traced as this body is small and loses its capacity for staining intensely at an early period. After the formation of the axial-filament, the outer centrosome wanders to the posterior end of the middle-piece, where presumably it remains. In the mature spermatozoön this centrosome probably serves as an "end-knob," as in the great majority of cases the connection between the two centrosomes, shown very clearly at the stages of Figs. 60-63, is broken before the spermatozoön becomes mature. I have never found the outer centrosome elongated as if it were in the process of division, neither have I found the ring-shaped structure around the axial-filament that is so conspicuous in the spermatids of *Salamandra* and of *Amphiuma*. The outer centrosome in *Bufo* behaves very much like the portion of the outer centrosome in the *Urodela* that remains at the posterior end of the middle-piece. This suggests the possibility that Hermann (21) is right in considering that the ring-shaped structure around the axial-filament in the spermatids of *Salamandra* is not derived from the centrosome. Herman states that the ring is formed from the mid-body of the last spermatocyte division;

but as Wilson (60) has pointed out, the figures given by McGregor and Meves show that the ring and the mid-body coexist in the young spermatid. Hermann's conclusion would seem, therefore, to be invalidated; but I can see no reason why the ring, if it is not derived from the centrosome, might not be formed from a part of the idiozome or, possibly, from a nebenkern.

There is as great a difference of opinion among investigators regarding the origin of the acrosome as there is concerning the formation of the middle-piece. Flemming (16) derives the apical portion of the spermatozoon of *Salamandra* from the achromatic substance of the nucleus; but this result is contradicted by the later researches of Meves (37) who finds, as does also McGregor, that this structure is formed from the idiozome. Broman (6) who has studied the formation of the spermatozoon in *Bufo igneus*, also derives the acrosome from the idiozome; but it is difficult to reconcile his description of the manner in which this formation takes place with that given by any other investigator of amphibian spermatogenesis. According to Broman, the idiozome becomes fixed at some point on the nuclear periphery and later the nucleus rotates in such a way that the idiozome is brought to the future anterior end of the spermatozoon. Whether the centrosomes, which are carried along with the idiozome, take any part in the formation of the acrosome is not stated.

Several investigators, among them Field (14), Niessing (42), and Platner (44), have maintained that the acrosome is formed from the spermatid centrosome; but such an origin of the acrosome has been denied by later workers on the same or on related forms, who have traced the centrosome into the middle-piece: Henking (19), Wilcox (59), and Paulmier (43), among others, have derived the acrosome in insects from a nebenkern: while Lenhossek (32) has described it in the rat as arising within the idiozome suddenly, as if it were a spontaneous thickening of the substance of the sphere from which it is later entirely separated, the sphere itself subsequently undergoing disintegration.

The young spermatids of *Bufo* contain in addition to the nucleus and the cytoplasm, a single centrosome surrounded by a clear vesicle, and a rounded, deeply staining body, the acroblast; there is no granular attraction-sphere in the cell, neither is there a nebenkern. As the acroblast is present in the cytoplasm of the primary spermatogonia and can readily be traced through all of the subsequent stages in the development of these cells, there is no possibility that it arises in the spermatid either from an idiozome, a mid-body, a centrosome, a nucleolus or a nebenkern.

Before the outer centrosome has reached the posterior end of the middle-piece (Fig. 63), the acroblast divides and one part migrates to the anterior end of the spermatid to form the acrosome. The fate of the acroblast that remains for a time in the posterior region of the spermatid I have not been able to ascertain. It is possible that this body has something to do with the formation of the marginal-filament or with the tail membrane. I cannot believe that it has persisted through all stages in the development of the spermatogonia unless it is to play some definite role in the formation of the spermatozoön.

An examination of the literature dealing with the spermatogenesis of amphibians shows that several investigators have found a homogeneous, rounded body in the cytoplasm that is similar in appearance to the acroblast in *Bufo*. Although Flemming and Meves makes no mention of such a body in the sperm-cells of *Salamandra*, Hermann states that in the spermatids of this amphibian "ein ovalärer Körper von dichter Consistenz findet sich; von einem hellen Hof umgeben." According to Hermann this body is formed in the spermatid from the remains of the achromatic spindle apparatus of the last spermatocyte division and soon disappears, being of secondary significance in the formation of the spermatozoön. Figures given by Kingsbury of the spermatogonia of *Desmognathus* show bodies in the cytoplasm resembling the acroblast, but no mention is made of these bodies in the text of the paper. The oval bodies found by Janssens in the spermatogonia of *Triton* are very like the acroblast in *Bufo*; but Janssens believes that these bodies are nucleoli extruded from the nucleus in order to nourish the cytoplasm. The behavior of the acroblast in *Bufo* would seem to invalidate the assumption that this body is an extruded nucleolus. As a rule, nucleoli that are ejected from the nucleus are dissolved at once or they lose their power of staining intensely and break up into granules which soon disappear. The acroblast, on the contrary, maintains its homogeneous appearance and stains very intensely during all stages of its development; it grows somewhat during the rest period of the cell, and then divides in the early prophase of mitosis, thus persisting as a definite structure in all generations of the sperm-cells.

In the cytoplasm of the spermatocytes and spermatids of various other groups of animals, small rounded bodies have been found that possibly are of the same character as the acroblast in *Bufo*. Since Benda (1) described the "chromatoid Nebenkörper" in the spermatocytes of mammals, a similar body has been found by other investigators of mammalian spermatogenesis. Lenhossek identifies the homogeneous body that is

present in the spermatocytes of the rat with the "chromatoid Nebenkörper" of Benda and he concludes, from its staining reactions, that it is an extruded nucleolus whose function may be to give up substances to the cytoplasm of the spermatid that are of use in the formation of the tail. Niessing also mentions the presence of a similar body in the spermatids of the guinea-pig, of the rat, and of the mouse. He traces this body back to the spermatocytes, although he fails to follow its history in the spermatids. Figures given by Niessing (Nos. 6, 21, 31, and 34) show that this body is in all respects like the acroblast in *Bufo*. His Fig. 34, showing a section of a young spermatid of a mouse, is especially interesting because in it this body is distinctly dumbbell shape, thus plainly indicating that it is in the process of division (compare with my Fig. 22, A). This stage given by Niessing is the one that I have been endeavoring in vain to find in the spermatids of *Bufo*; for although I have found numerous instances in which two acroblasts were lying close together, I have never succeeded in finding the actual division stage. It is a rather significant fact that Niessing is one of the few investigators who have stated that the acrosome is derived from a spermatid centrosome.

The acroblast in the young spermatids of *Bufo* might readily be mistaken for a large centrosome if one had not traced its development up to this stage. I am inclined to think, therefore, that the presence in the spermatids of other forms of a body similar in nature to the acroblast in *Bufo* may have caused many of the conflicting results that have been obtained by investigators regarding the origin of the acrosome. On this assumption it is probable that the "centrosome" described by Niessing, Field, Platner, and others as forming the acrosome, will be identified with the acroblast that migrates to the anterior end of the spermatid of *Bufo*; the real centrosome being found in the posterior end of the sperm-head or in the middle-piece. Platner's figures of the spermatids of a butterfly (Figs. 6-9) readily lend themselves to this interpretation, as his "centrosome" is much larger than that usually found in the spermatids of insects.

Besides the work of Platner, Henking's (19) figures of the spermatids of *Pyrrhocoris apterus* strongly suggest that a body like the acroblast may be present in the sperm-cells of some of the invertebrates as well as in those of the vertebrates. In addition to a nebenkern and a mitosome, both of which he derives from the remains of the spindle of the last spermatocyte division, Henking figures in the cytoplasm a small rounded, deeply staining body (Figs. 68, 69, 71, 72, 77-80). In the young spermatid this body seems to have no definite location, but in later

stages (Figs. 79, 80), it is found at the anterior end of the sperm-nucleus. In describing the origin of the acrosome, Henking states that the mitosome divides and that a portion of it becomes infected by an "unzweifelhaft chromatisches Punktchen" so that the whole body stains like chromatin and then wanders to the anterior end of the spermatid to form the acrosome. Henking makes no mention in the text of his paper of the small bodies in the cytoplasm, and I am strongly inclined to think that his "infected mitosome" may prove to be an acroblast.

In this connection observations made by Foot and Strobell (17) are of interest. These investigators state that in the spermatozoön of *Allolobophora foetida* there are three centrosome-like structures, one at the base of the spine, one at the anterior, one at the posterior end of the middle-piece. It is perhaps possible that here, too, the centrosome-like body at the apex of the spermatozoön is derived from an acroblast.

In all of the cases in which a body similar to the acroblast in *Bufo* has been described or figured, this structure has the same characteristics: it is round or oval and somewhat larger than a centrosome; it always appears homogeneous and stains very intensely; it is usually surrounded by a clear area. It hardly seems as if bodies of unlike nature would have the same characteristics in the spermatids of such varied forms as the insects, the amphibians, and the mammals. Few of the investigators who have mentioned the presence of this body in the spermatids have traced it back to the spermatocytes; and none of them have followed its history in the spermatid. I feel confident that further research will show that in many, if not in all, of the cases mentioned above, the rounded, homogeneous body in the spermatids will be found to be of the same nature and significance as the acroblast in the spermatids of *Bufo*.

As the formation of the acroblast in the primary spermatogonia is as yet obscure, any conjecture I may make as to the origin of this body will be purely tentative. Judging from its staining reactions the acroblast is not extruded chromatin; and the behavior of this body lessens the probability that it is a nucleolus. I am strongly inclined to the opinion that the acroblast is a purely cytoplasmic product, formed possibly, from a condensation of a portion of the attraction-sphere at an early period in the history of the primary spermatogonia. If such proves to be the case, then in the spermatozoön of *Bufo* the acrosome has practically the same origin as the acrosome in the spermatozoön of *Salamandra*, *Amphiuma*, and *Bombinator*.

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EXPLANATION OF FIGURES ON PLATES I-III.

All figures were drawn with the aid of a camera lucida under a Zeiss apoc. 1.5 mm., oc. 8. The following abbreviations are used in lettering the figures: *F. C.*, follicle cells; *A.*, acroblast; *C.*, centrosome; *Ac.*, acrosome; *M. P.*, middle-piece.

EXPLANATION OF PLATE I.

FIG. 1. Resting primary spermatogonium surrounded by follicle cells.

FIGS. 2-3. Early prophase of mitosis in a primary spermatogonium. All 24 chromosomes are shown.

FIG. 4. Equatorial section of the spindle in a primary spermatogonium during metakinesis.

FIG. 5. Longitudinal section of the spindle in a primary spermatogonium during metakinesis.

FIG. 6. A secondary spermatogonium during the resting stage.

FIG. 7. Early prophase of mitosis in a secondary spermatogonium.

FIG. 8. Longitudinal section of the spindle in a secondary spermatogonium during metakinesis.

FIG. 9. Anaphase of mitosis in a secondary spermatogonium.

FIG. 10. Young primary spermatocyte before the formation of the nuclear reticulum.

FIGS. 11-14. Growth stages of the primary spermatocytes.

FIG. 15. The primary spermatocyte at the end of the growth stage. The nucleus contains a spireme that appears to be continuous.

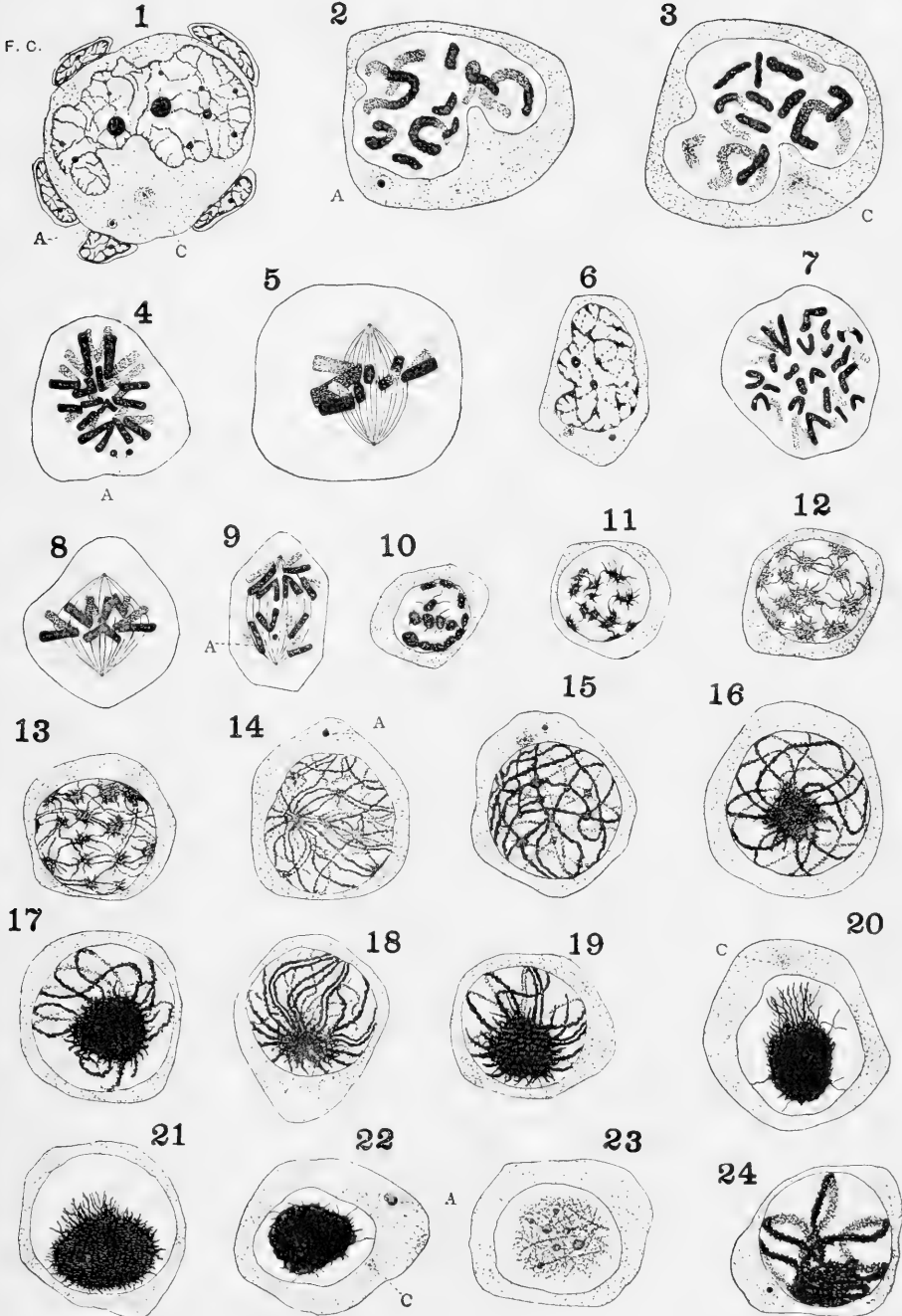
FIGS. 16-21. Stages showing the gradual condensation of the chromatin that precedes synizesis.

FIG. 22. Synizesis stage in the primary spermatocytes. The mass of chromatin is stained black with iron-hæmatoxylin.

FIG. 23. Same stage as the preceding, showing the appearance of the mass of chromatin when the greater part of the iron-hæmatoxylin has been extracted.

FIGS. 24-25. Post-synizesis stages in the primary spermatocytes. The chromatin is being evolved in the form of a continuous spireme.

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EXPLANATION OF PLATE II.

FIGS. 24-25. Post-synizesis stages in the primary spermatocytes. The chromatin is being evolved in the form of a continuous spireme.

FIGS. 26-27. Continuous spireme found in the primary spermatocytes at the end of the synizesis stage.

FIG. 28. Beginning of the segmentation of the continuous spireme into the reduced number of chromosomes.

FIG. 29. A slightly later stage than that shown in Fig. 28. The spireme is being constricted into chromatin segments.

FIG. 30. The stage succeeding that shown in Fig. 29. The chromosomes are nearly separated and they are distinctly dumbbell shape.

FIGS. 31-33. Formation of ring-shaped chromosomes in the early prophase of mitosis.

FIG. 34. Primary spermatocyte in which the chromosomes are in the form of bead-like segments before they have separated. Such segments later form rings.

FIG. 35. Primary spermatocyte in which the spireme has segmented into rectangular shaped blocks.

FIG. 36. Formation of ring-shaped chromosomes from the spireme in the early prophase of mitosis.

FIG. 37. Primary spermatocyte in the early prophase of mitosis. The nucleus contains dumbbell-shaped chromosomes that are connected end to end by linin fibers.

FIG. 38. About the same stage as Fig. 37. The nucleus contains both dumbbell and ring-shaped chromosomes.

FIG. 39. Later prophase of mitosis. Chromosomes of different shapes are scattered throughout the nucleus.

FIG. 40. Ring-shaped chromosomes in the nucleus previous to the formation of the first maturation spindle.

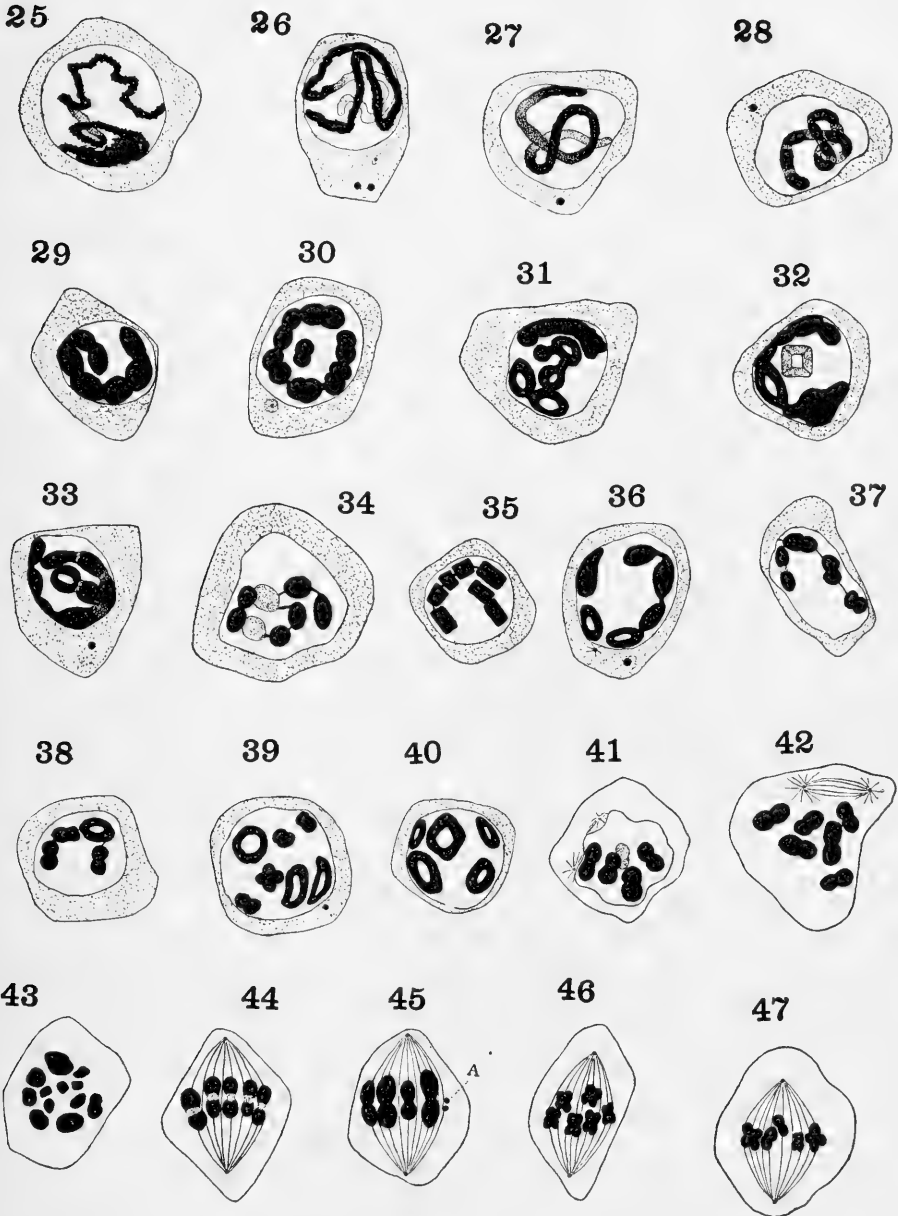
FIGS. 41-42. Stages in the formation of the first maturation spindle.

FIG. 43. Equatorial plate of the first maturation spindle showing 12 chromosomes of different sizes.

FIGS. 44-45. Longitudinal sections of the first maturation spindle during metakinesis.

FIG. 46. Same stage as the preceding. The chromosomes have formed typical tetrad groups.

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EXPLANATION OF PLATE III.

FIG. 47. Metakinesis of the first maturation division. Chromosomes of different types on the same spindle.

FIG. 48. Same stage as the preceding, showing the great difference in the size of the chromosomes.

FIG. 49. Early anaphase of the first maturation mitosis.

FIGS. 50-52. Anaphase of the first maturation mitosis.

FIG. 53. Massing of the chromosomes at the spindle poles in the late anaphase of the first maturation mitosis.

FIG. 54. Secondary spermatocyte. Division of the centrosome preparatory to the formation of the second maturation spindle.

FIG. 55. Metaphase of the second maturation mitosis, showing an amorphous mass of chromatin at the equator of the spindle.

FIG. 56. Equatorial plate of the second maturation spindle. Only nine of the twelve chromosomes are shown.

FIG. 57. Early anaphase of the second maturation mitosis.

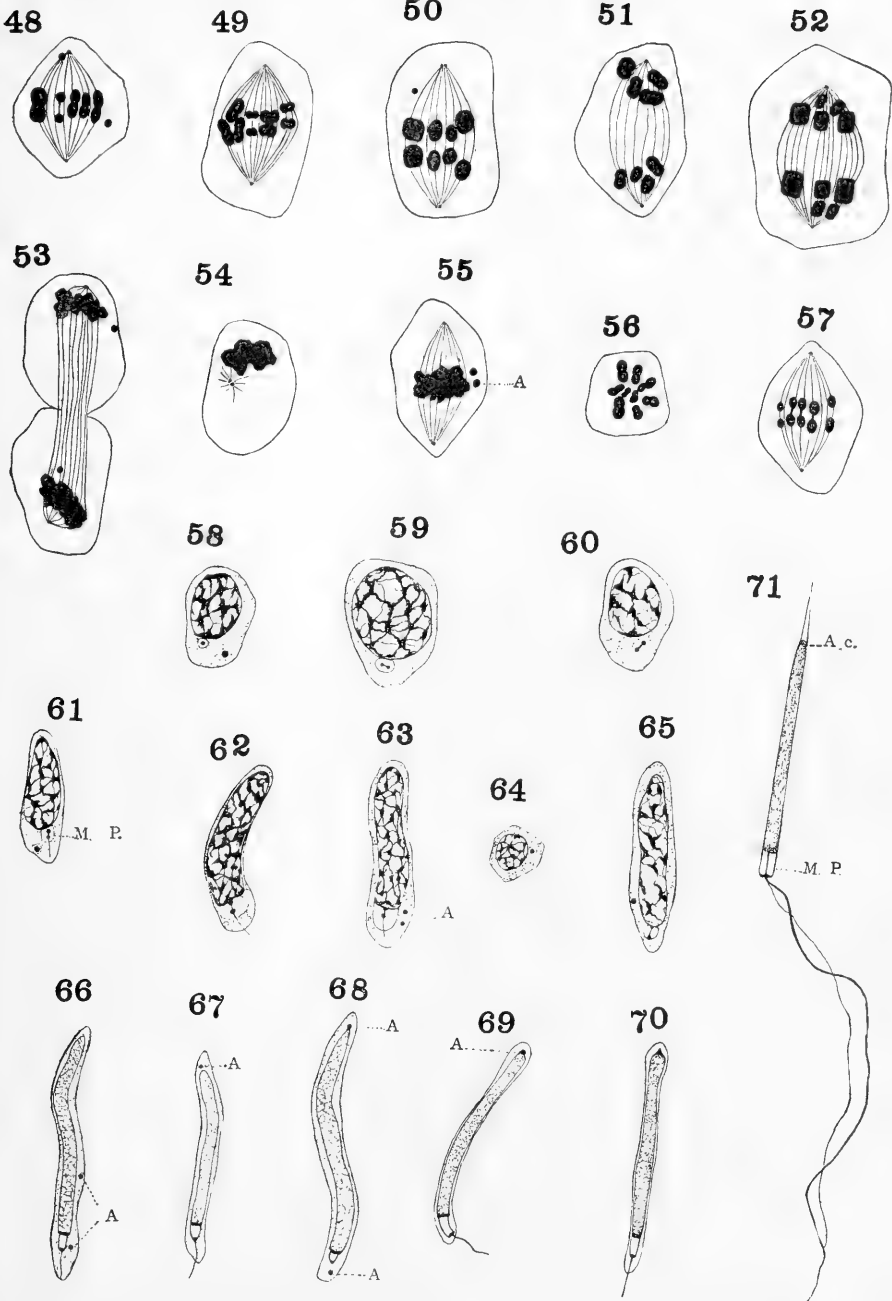
FIG. 58. Very young spermatid showing the single centrosome and the acroblast.

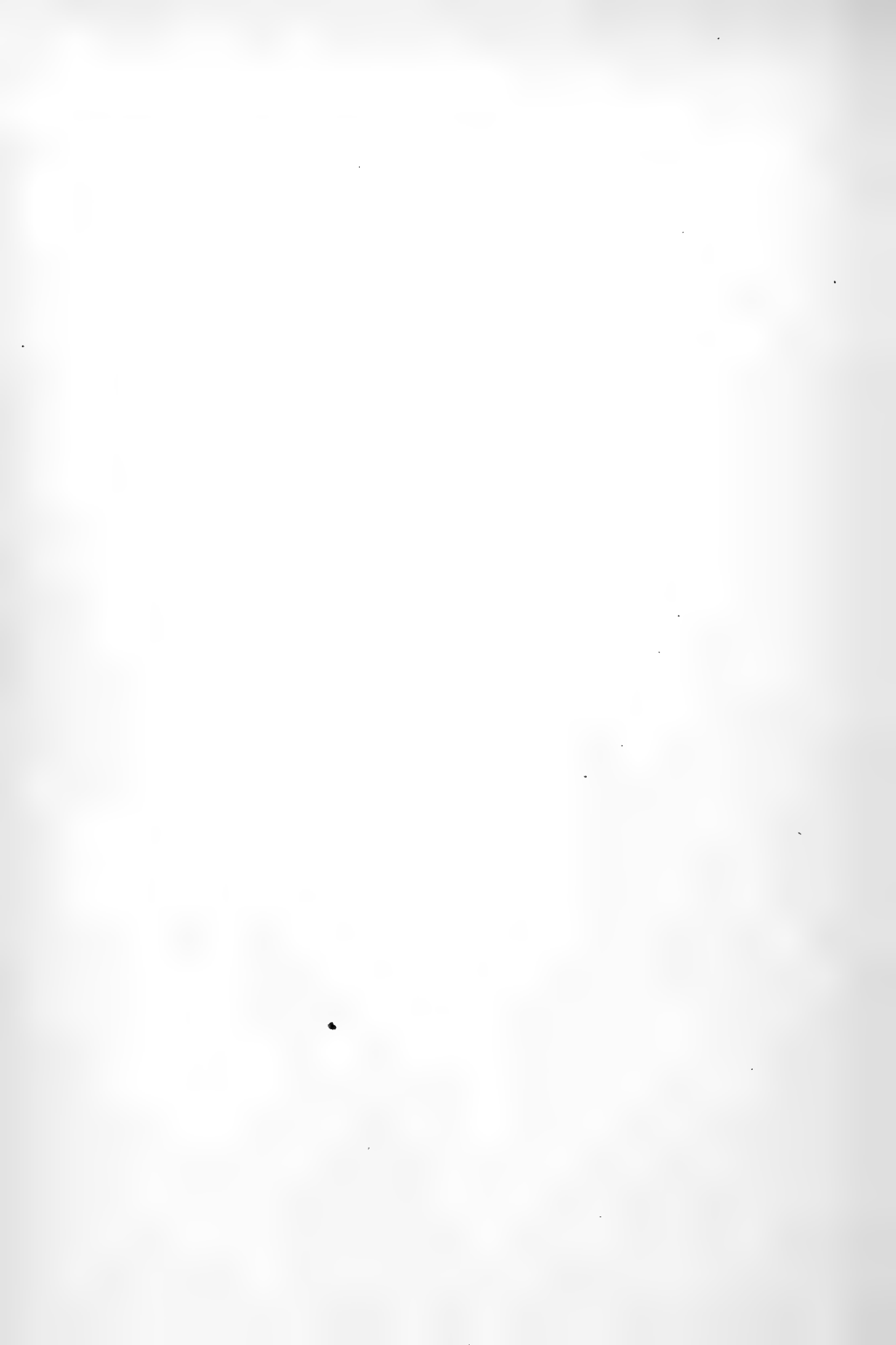
FIG. 59. Young spermatid. The centrosome has divided and the surrounding vesicle has come in contact with the nuclear membrane to form the middle-piece.

FIGS. 60-70. Stages in the development of the spermatid into the spermatozoön.

FIG. 71. Mature spermatozoön stained lightly with iron-hæmatoxylin.

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THE VASCULAR SUPPLY OF THE PLEURA PULMONALIS.

BY

WILLIAM SNOW MILLER.

From the Anatomical Laboratory of the University of Wisconsin.

WITH 12 TEXT-FIGURES.

In a paper presented before the Association of American Anatomists in 1905 (9 e), I described the vascular supply of the bronchi and the relation which the bronchial circulation bore to the pulmonary. I purposely refrained from making any statements in regard to the distribution of the bronchial artery to the pleura for I wished to make a more extended study of the problem.¹

That the histological structure of a given organ may differ in animals of different species, and even in different animals of the same species, is coming to be accepted more and more by investigators and it seems quite probable that eventually each animal will have to be studied independently. In no place is this fact so forcibly presented as in the excellent series edited by Opper (10).

Because a given structure, or relation of structures, is found in, for example, rabbits, it is no criterion that the same structure, or relation of structures, will be found in rats, and the inverse is true. We may, however, find a type which, modified to a greater or less degree, runs through an extended series of animals of different species.

In previous communications (9) I have stated that the pleura did not receive its vascular supply from the bronchial artery. I fear I failed to impress on the minds of my readers that this statement was made in regard to the dog; for some of my co-workers, notably Königstein (7), have taken me to task for this statement. I myself made the mistake of thinking that the arrangement found in the dog's pleura would hold true elsewhere and so found myself at variance with Küttner (8) and Zuckerkandl (15) who described branches of the bronchial artery as being distributed to the pleura of the human lung. That they were correct in so stating, the present paper will show and, at the same time, I trust, will bring out some facts hitherto overlooked.

¹ I wish to express here my thanks to Professor F. P. Mall and Dr. A. W. Meyer, of the Johns Hopkins Medical School for their generous assistance in procuring the necessary material for this study.

METHOD OF STUDY.

In my study of the blood-vessels, I have used as a means of differentiating the various systems of vessels a 20 per cent solution of gelatine containing colored granules. If a Prussian blue or carmine gelatin be used, one can not be sure that a given network belongs to any individual system of vessels. If, on the other hand, the injection mass be charged with granules of such size that they will not readily pass the capillaries, then all the derivatives of the vascular trunk injected will stand out sharply and distinctly. I can not but think that this method of injection gives more exact results than the former. Take for example a lobe of a dog's lung. A cannula is tied in the branch of the pulmonary artery which supplies the lobe; a second cannula is tied in the branch of the pulmonary vein coming from the lobe. A 10 per cent solution of Prussian blue gelatin is first injected into the artery until the lobe is thoroughly blue and the mass runs freely from the open cannula tied in the vein. The injection is now discontinued and a flask of 20 per cent gelatin containing vermilion granules is connected with the pulmonary artery and a second flask of 20 per cent gelatin containing Ultra-marine blue granules is connected with the pulmonary vein. These two granular masses are injected simultaneously. The result is that the capillaries are filled with a transparent blue mass; while the pulmonary artery and its branches are opaquely red; the pulmonary vein and its branches are opaquely blue. There is no mistaking one set of vessels for the other. It is by means of various modifications of this method that the present study has been made.

Dog.—In the dog, repeated injections of a granular mass, prepared as above, directly into the bronchial artery have failed to demonstrate any branches passing to the pleura. Only at the hilus have I seen short twigs, two to five millimeters in length, given off from the main stems of the bronchial artery, these were distributed to the connective tissue about the hilus of the lung and did not extend to the pleura. I have also failed to find any branches of the bronchial artery extending from the depth of the lung to the pleura. It seems quite probable that the absence of pronounced connective-tissue septa in the dog's lung is an important factor in the arrangement of the bronchial artery.

Küttner (8) has described branches of the bronchial artery as being distributed directly to the pleura and anastomosing with the pulmonary artery. My injections have failed to confirm his results.

I have previously stated in regard to the pulmonary artery (9), that,

“as a rule no artery passes to the periphery of the lobule.” The significance of the exceptions I failed for some time to appreciate.

If sections be made at right angles to the pleura of a dog's lung, in which the blood-vessels have been injected as above indicated, here and there, it will be seen that a branch of the pulmonary artery extends beyond the plane at which the others break up into capillaries. When this branch reaches the pleura it bends upon itself, gives off radicles to the pleura, then passes towards the center of the lobule for a short distance where it breaks up into capillaries (Fig. 1, *P. A.*).

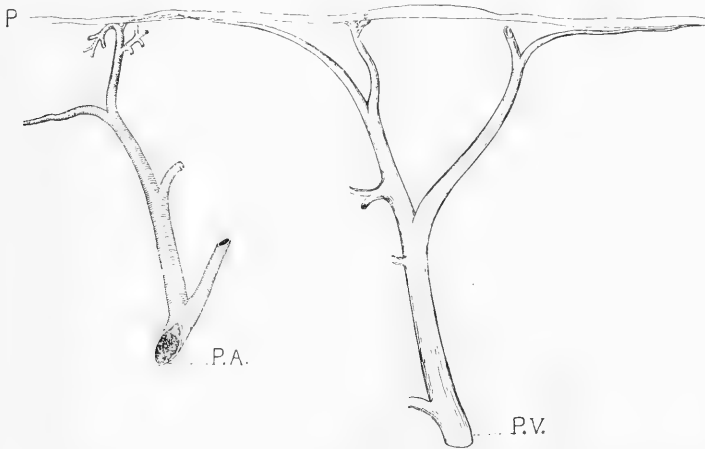


FIG. 1. Section of the lung of a dog taken at right angles to the pleura. Camera lucida tracing showing the relation of the blood-vessels to the pleura. The parenchyma of the lungs is omitted from the drawing. *P* = pleura pulmonalis. *P. A.* = pulmonary artery, one branch of which can be seen arching under the pleura and sending a small radicle to the pleura. *P. V.* = pulmonary vein, one branch of which originates from the network into which the pleural radicle of the pulmonary artery breaks up. $\times 50$.

In sections, taken parallel to the pleura and which include the pleura, it will be seen that only a small portion of the pulmonary artery approaches the surface and that this is situated nearly equi-distant from the branches of the pulmonary vein (Fig. 2).

From the branches of the pulmonary artery, which extend to the pleura, a system of capillaries is formed which, in turn, empties into the radicles of the pulmonary vein (Fig. 1).

The pulmonary veins, on the other hand, as they approach the pleura branch repeatedly, and all of these branches run for some distance in, or just beneath, the pleura (Figs. 1, 2).

The present study of the pleural blood-vessels has brought out one point which I overlooked at the time I studied the lymphatics of the pleura (9 c), and which I do not find mentioned or illustrated in any previous contribution to the histology of the lung, namely, a distribution of blood-vessels to the lymphatics.



FIG. 2. Surface view of the pleura of the lung of a dog. *P. A.* = pulmonary artery. *P. V.* = pulmonary vein. The pulmonary artery is seen arching under the pleura and giving off branches to the pleura. The characteristic branching of the pulmonary veins is also shown. Camera lucida tracing. Details omitted. $\times 50$.

From the radicles of the pulmonary artery, which extend to the pleura, branches are given off which arrange themselves parallel to the course of the lymphatics. Generally, there is a blood-vessel on each side of a lymphatic; these are connected at intervals by transverse branches which run external to the lymphatic. There is thus formed a network of blood vessels the mesh of which is roughly rectangular, the long axis of the rectangle being placed at right angles to the course of the lymphatic (Fig. 3).

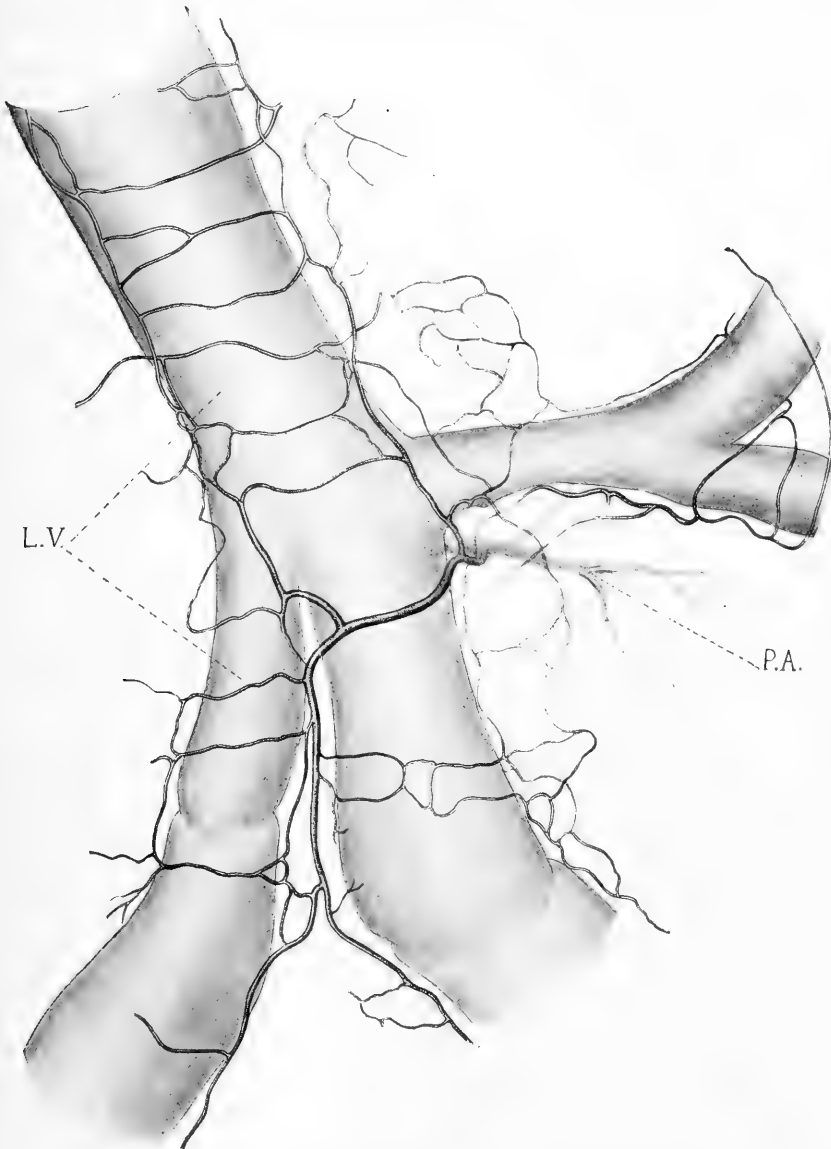


FIG. 3. From a section taken parallel to, and including, the pleura of the lung of a dog. Thickness of section 100 micra. The lymph vessels are fully distended. *L. V.* = lymph vessel. *P. A.* = pulmonary artery, situated beneath the pleura. A radicle can be seen arising from the artery and giving origin to the network of vessels about the lymphatics. $\times 50$.

The under surface of the lymphatics is apparently supplied by the capillary network of the air sacs (*sacculi alveolares*), over which they run, since encircling loops are rarely present in my preparations.

When the lymphatics are collapsed, the relation of the blood-vessels to the lymph vessels is not so apparent; it is only when the lymph vessels are distended that the relationship is best seen. On the other hand, having become familiar with this relationship, the situation of the lymphatics can be made out from the course of the blood-vessels, when they are well injected.

The capillary network of blood-vessels, which belongs to the remaining portion of the pleura, though continuous with that over the lymphatics, differs in the size and shape of its mesh and is readily distinguished from that over the lymphatics (Fig. 3). The entire network of pleural capillaries discharges its blood into the radicles of the pulmonary vein.

That which I have figured in a previous contribution (9 c) as the capillary network of the pleura belongs to the peripheral walls of the *sacculi alveolares* and not to the pleura proper. The mesh of the system of capillaries belonging to the *sacculi alveolares*, just beneath the pleura, is much coarser than that of the *sacculi alveolares* in the depth of the lung; that belonging to the pleura itself is still coarser.

Sheep.—In the sheep, the pleura receives its vascular supply in a manner quite different from that in the dog. Though thus far I have failed to demonstrate the bronchial artery as being distributed to the pleura of the dog, the first injection made showed a rich distribution of the bronchial artery to the pleura of the sheep.

From the hilus of each lung, and from a main trunk which runs along the pulmonary attachment of the *ligamentum pulmonale*, branches of the bronchial artery can be seen passing to all the lobes of the lung (Fig. 4). In general, the main branches on the *facies mediastinalis* and *facies diaphragmatica* may be said to run parallel to each other. These main branches are connected by numerous anastomoses and there is thus formed a coarse plexus of vessels the mesh of which is occupied by a network of still finer vessels.

Figure 4 shows several branches of the bronchial artery entering the *incisura interlobaris*. These branches are distributed to what may be called the *facies interlobaris* of the superior and inferior lobes of the lung.

The *facies costalis* (Fig. 5) receives its vascular supply in quite a different manner. Branches of the bronchial artery extend over the *margo inferior* and the *margo anterior* to the *facies costalis* and there break up

into finer ramifications. Branches also arch over dorsally from the facies diaphragmatica and facies mediastinalis. One or more of the branches which enters the incisura interlobaris extends through the incisura and reaches the facies costalis.

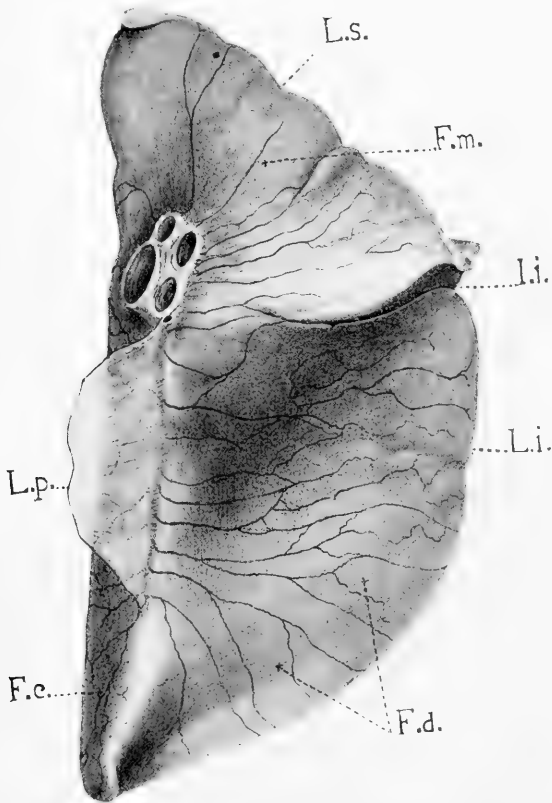


FIG. 4. Left lung of a sheep partially distended. Two-thirds natural size. The lung is seen from below and somewhat from the mesial surface. *F. d.* = facies diaphragmatica. *F. m.* = facies mediastinalis. *F. c.* = facies costalis. *L. i.* = incisura interlobaris. *L. p.* = ligamentum pulmonale. *L. s.* = lobus superior. *L. i.* = lobus inferior. The cut bronchus and vessels are readily recognized at the hilum. From the hilum and from the branch situated along the attached border of the ligamentum pulmonale the branches of the bronchial artery (solid black lines) can be traced to their distribution on both lobes of the lung.

This arrangement leaves the center of the facies costalis without any direct branches of the bronchial artery. The deficiency is supplied, however, by branches of the bronchial artery which come to the surface from the depths of the lung.

Along the *margo inferior* certain of the arteries instead of arching over the margin turn and enter one of the septa of the lung and, passing along this, appear on the *facies costalis*. Several of such examples are shown in Fig. 5.

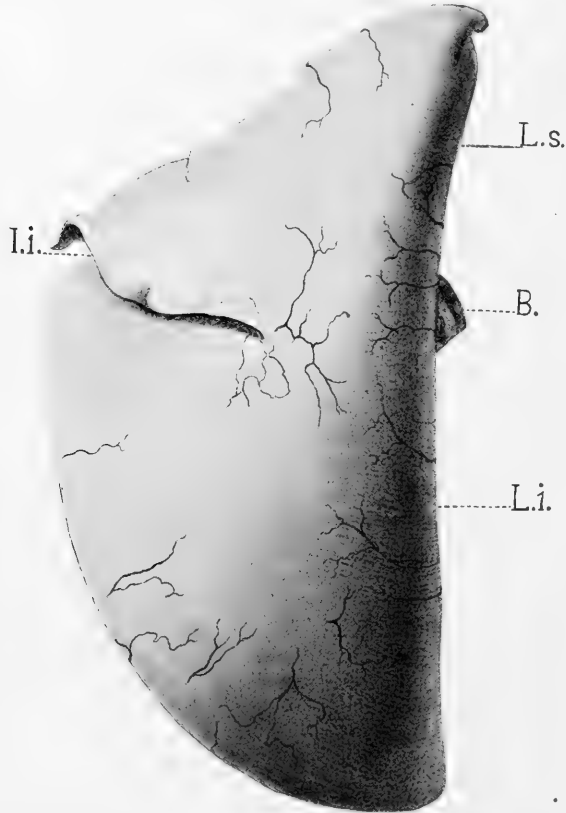


FIG. 5. *Facies costalis* of the lung shown in Fig. 4. *B.* = bronchus sinister. *L. s.* = lobus superior. *L. i.* = lobus inferior. *I. i.* = incisura interlobaris. The branches of the bronchial artery (solid black lines) can be seen arching over the margins of the lung and coming up from the depth of the lung. Many of the vessels have been omitted; only those easily seen with the unaided eye are shown. Two-thirds natural size.

Not only is the pleura in the sheep supplied by branches of the bronchial artery, which pass directly to it, but it is also supplied indirectly by branches of the bronchial artery which pass into the substance of the lung at the hilus and, after giving off branches to the bronchi and to the connective-tissue septa, send radicles to the pleura. These radicles anas-

tomose with the network already described. Just mesial to the termination of the incisura interlobaris a very prominent branch can be seen in Fig. 5 which comes from the depth of the lung.

Fig. 6 shows a combination drawing of three consecutive sections taken perpendicular to the pleura. A branch of the bronchial artery can be seen

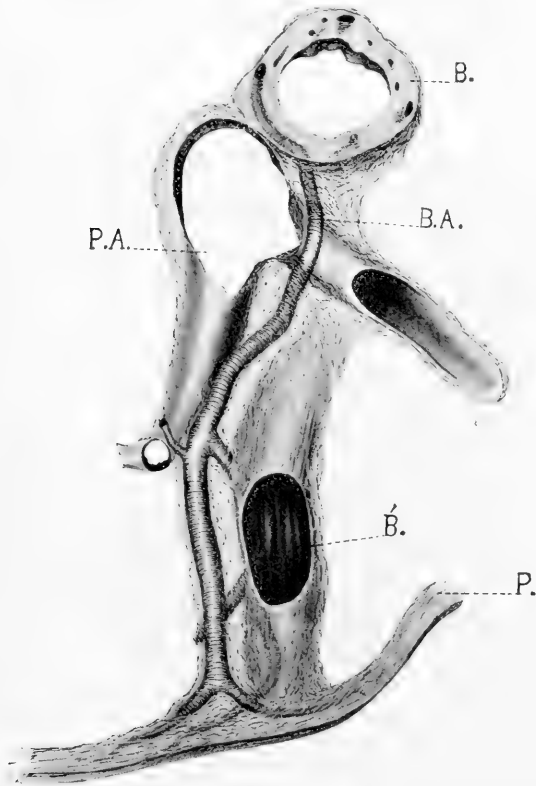


FIG. 6. Lung of the sheep. Combination of three successive sections taken perpendicular to the pleura. *B.* = bronchus in the wall of which sections of the bronchial artery are seen. *B'* = a branch of *B* which here runs a short distance beneath the surface. *B. A.* = bronchial artery. *P.* = pleura. *P. A.* = pulmonary artery. For further description, see text. $\times 25$.

following a bronchus and one of its branches, which here runs a short distance beneath the pleura, then, leaving the bronchus, it joins the network of vessels derived from the branches of the bronchial artery which pass directly to the pleura.

The vascular network about the lymphatics in the sheep's pleura is

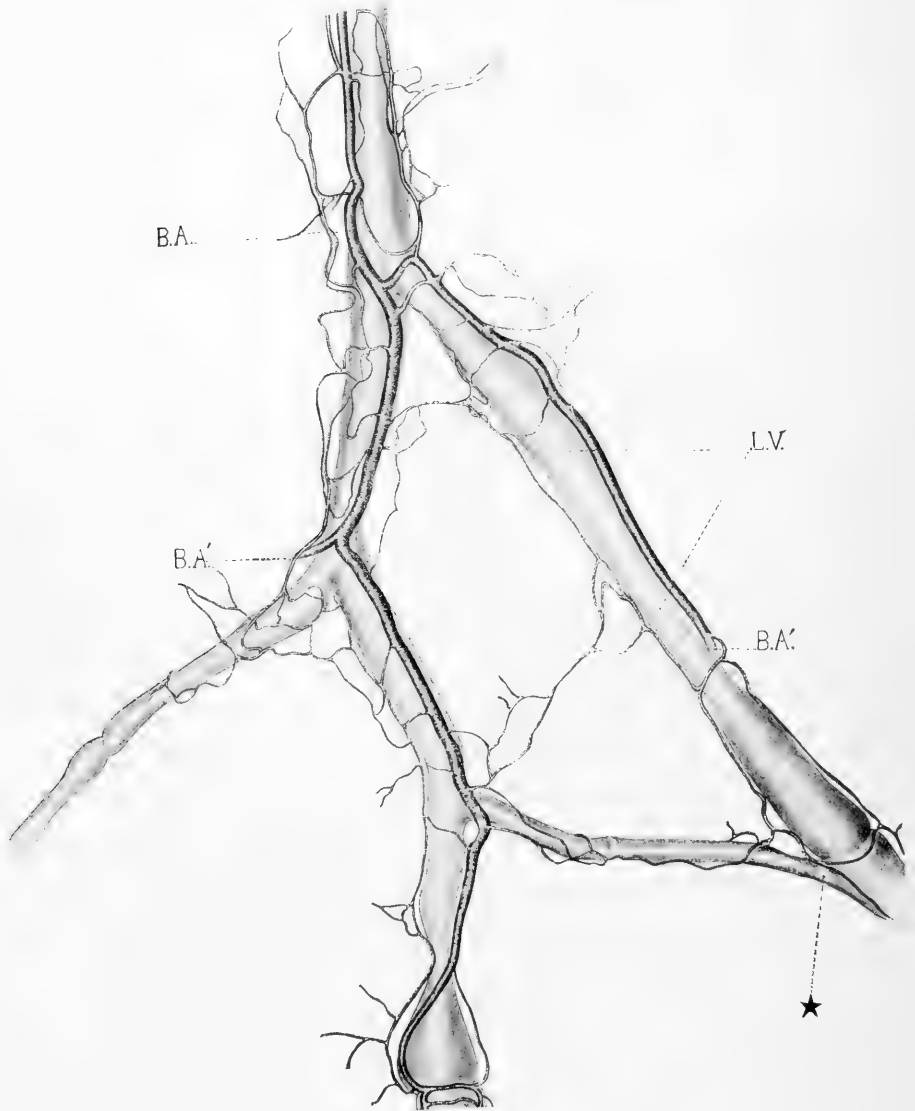


FIG. 7. From a section taken parallel to, and including, the pleura of the lung of a sheep. Thickness of section 200 micra. The lymphatics are partially collapsed. *B. A.* = bronchial artery. *B. A'* = points at which the pleural and pulmonary branches of the bronchial artery anastomose. *L. V.* = lymph vessel; in several places the valves are plainly visible. * = point at which a deep lymphatic joins the superficial network. $\times 33\frac{1}{2}$.

derived from the bronchial artery. There is usually a main arterial trunk on one side of a lymphatic and from this branches are given off which pass over and also beneath the lymphatic to the opposite side where they join to form a smaller vessel (Fig. 7). There is thus formed an arterial plexus which is placed external to the lymphatic. Along smaller lymphatics the two parallel arteries are usually of the same size. Here and there, branches can be recognized which connect the pleural vessels with those within the lung (Fig. 7).

In sections taken perpendicular to the course of the lymphatics it can be seen, in lungs in which the bronchial and pulmonary systems have

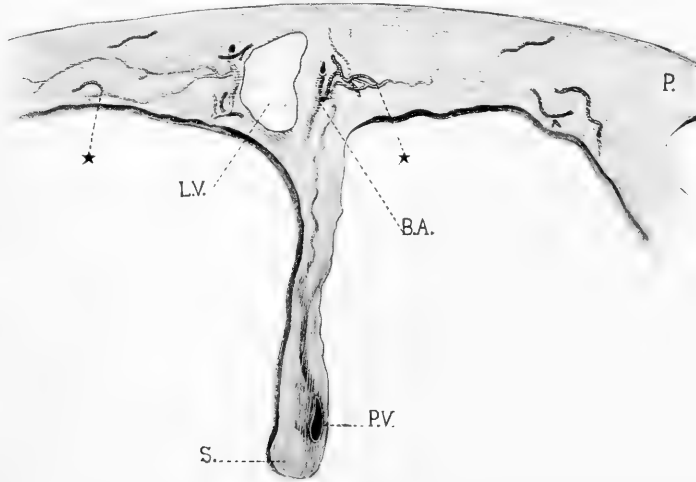


FIG. 8. Lung of sheep. Section taken at right angles to the pleura. Semi-diagrammatic. The outline, size, and position of vessels drawn by means of the camera lucida. *P.* = pleura. *S.* = septum. *B. A.* = bronchial artery. *L. V.* = lymph vessel cut obliquely. *P. V.* = pulmonary vein. ** = places where the capillaries from the bronchial artery join the radicles of the pulmonary vein. From one of these points the course of the venous radicle can be traced along a septum to a main branch of the pulmonary vein. $\times 50$.

been injected with differential masses, that from the plexus about the lymphatics capillaries are given off which unite to form radicles and that these radicles join branches of the pulmonary vein which are situated in the septa of the lung (Fig. 8). A few of the capillaries enter directly into the capillary network of the sacculi alveolares which are situated just beneath the pleura.

That the plexus about the lymphatics is to be considered as arterial is shown by granular injecting masses completely filling the plexus while

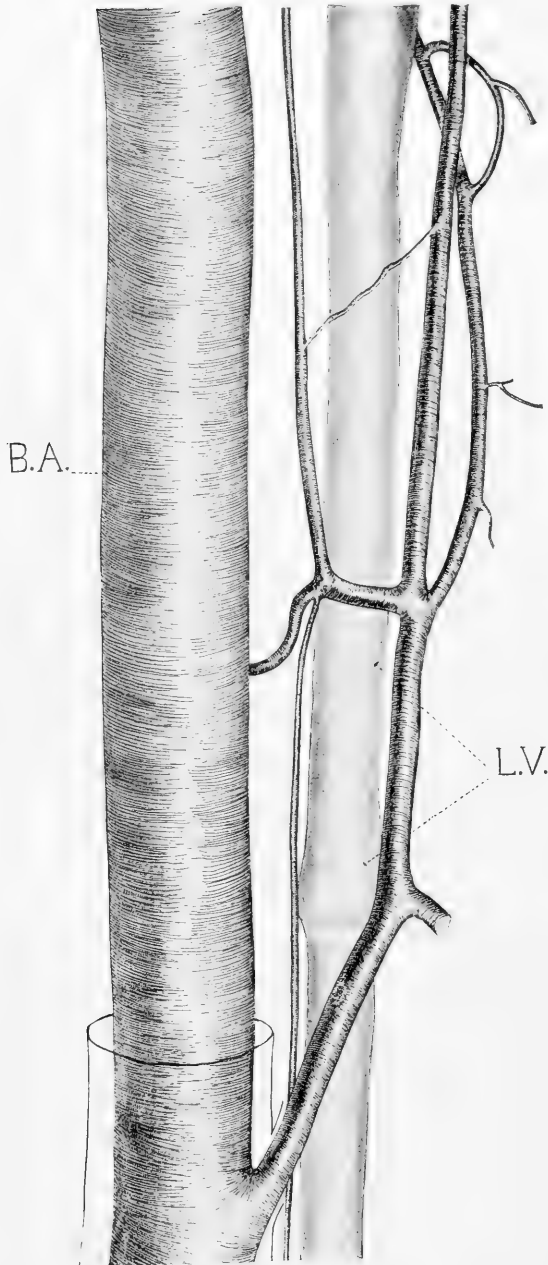


FIG. 9. From a section taken parallel to, and including, the pleura of the lung of a horse. Thickness of section 200 micra. The lymphatics were collapsed and difficult to follow. The lymphatic represented in the illustration was selected because it followed one of the main branches of the bronchial artery and gave an excellent opportunity of showing the large size of the bronchial vessels in the pleura of the lung of the horse. In the lower portion of the illustration the thickness of the arterial wall is shown in outline. B. A. = bronchial artery. L. V. = lymph vessel. $\times 50$.

the same mass does not enter the capillaries which go to join the radicles of the pulmonary vein.

At the hilus of the lung I have occasionally seen pleuro-bronchial veins, which join the true bronchial veins, coming from the first, and sometimes from the first two, divisions of the bronchi within the lung (9e).

Horse.—In the horse branches of the bronchial artery are distributed directly to the pleura and also reach the pleura indirectly; that is, after having first traversed the substance of the lung. The later branches, on reaching the pleura, anastomose with those that go directly to the pleura.

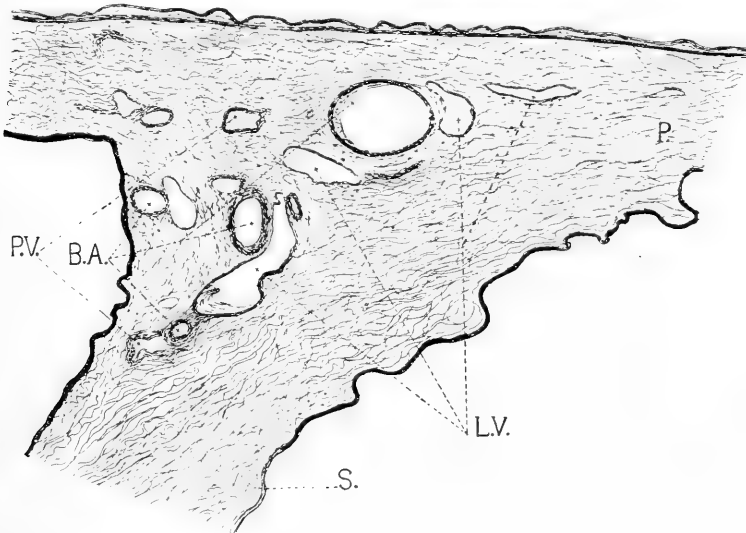


FIG. 10. Section taken at right angles to the pleura of the lung of the horse. Semi-diagrammatic. The outline of the section, the size and position of the vessels were drawn by means of the camera lucida. *P.* = pleura. *S.* = septum. *B. A.* = bronchial artery. *L. V.* = lymph vessels. *P. V.* = pulmonary vein. $\times 50$.

The main branches of the bronchial artery, which are directly distributed to the pleura, are of large size, much larger than in any other lung I have studied; their walls are very thick and contain a large amount of smooth muscle.

In the sheep and in man the pencil line with which the injected lumen of the arteries were outlined under the camera included at the same time the walls of the vessels; this is not the case in the horse. Fig. 9 shows one

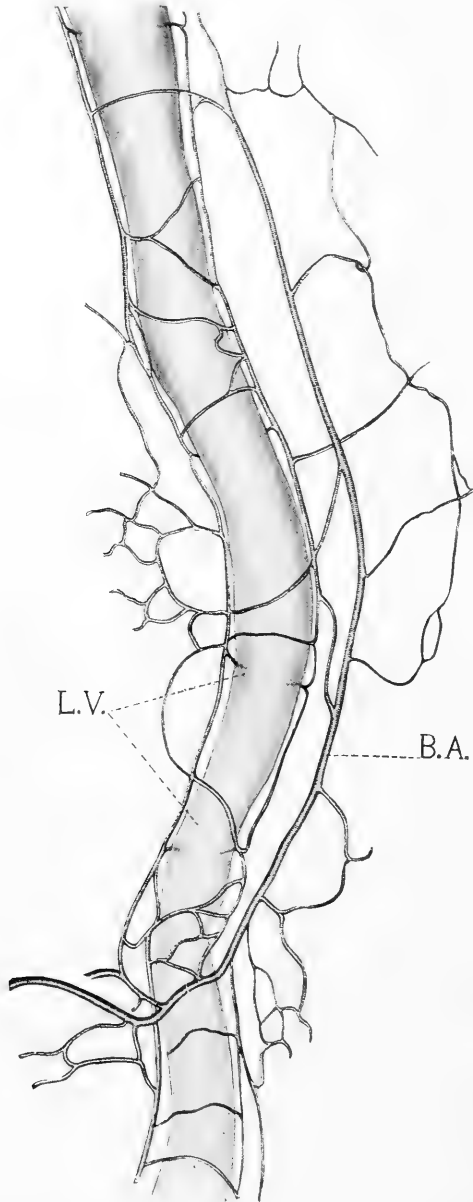


FIG. 11. A lymphatic and its accompanying blood-vessels from the pleura of the lung of man. The lymphatic is partly collapsed. The encircling network of blood-vessels is well shown. B. A.=bronchial artery and its branches. L. V. = lymph vessel. $\times 50$.

of the main branches near the margo inferior of the right lung and, consequently, near its final breaking up into smaller vessels. This branch can be readily compared with the branches of the bronchial artery in the pleura of the sheep and man (Figs. 7 and 11), because they are drawn on the same scale. In the lower part of Fig. 9 the contour lines indicate the thickness of the arterial walls; in the remainder of the drawing only the injected lumen of the artery is indicated.

The lymphatics in the pleura of the horse, like those of the sheep, are supplied by the bronchial artery. In Fig. 9 the encircling network of blood-vessels is not as pronounced as in the sheep or in man. The illustration was selected more especially to show the large size of the

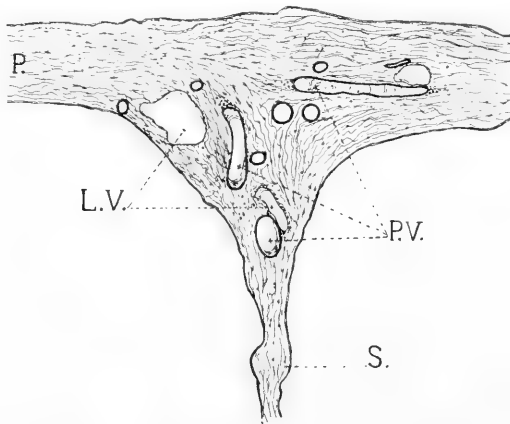


FIG. 12. Section taken at right angles to the pleura of the human lung. Semi-diagrammatic. The outline of the section, the size and position of the vessels were drawn by means of the camera lucida. P. = pleura. S. = septum. L. V. = lymph vessels. P. V. = pulmonary vein. The branches of the bronchial artery are represented by heavy black lines. $\times 50$.

blood-vessels. In Fig. 10 the relation between the lymph and blood-vessels is well demonstrated and one can see that there is in the horse the same relationship between the two systems as in the sheep and in man. In the pleura of the horse there is a thick, dense layer of elastic fibers overlaying the blood- and lymph-vessels which makes it difficult to obtain clear surface views of the finer blood-vessels and transparent lymph vessels.

Man.—In no instance have I been able to demonstrate in the human pleura the long, nearly parallel, branches of the bronchial artery which are such a prominent feature in the pleura of the sheep and the horse:

only here and there, have I found branches of the bronchial artery passing directly to the pleura. It does not seem to me that this is due to faulty injections, for I have always been able to obtain a well-filled system of vessels in the pleura.

I have always found that the branches of the bronchial artery which reach the pleura came from the depth of the lung and that they are larger in proportion and more branched than in the sheep or in the horse.

Küttner (8) has given an excellent illustration in his Fig. 7 of the artery spreading out in the pleura of the lung of an infant. The figures which I have obtained in the adult agree with his of the child.

It appears therefore, that in the sheep and in the horse the type of the distribution of the bronchial artery to the pleura is different from that in man. In the two former, the supply is mainly direct, in the latter, indirect.

The lymphatics of the pleura in man, like those of the sheep and the horse, are surrounded by a network of blood-vessels which is derived from the bronchial artery (Fig. 11). This network seems to be finer and there are more encircling branches than is the case with either the sheep or the horse, though some of my preparations of the sheep's pleura show a very complete network.

The capillary network into which the bronchial artery breaks up in the pleura gives rise to radicles which join the pulmonary vein. These venous radicles are larger in the human pleura than in either the sheep or the horse (Fig. 12).

ANASTOMOSES BETWEEN THE BRONCHIAL ARTERY AND THE PULMONARY ARTERY.

The question naturally arises: What constitutes an anastomosis? In general, we understand by the term, as applied to the vascular system, a communication between two different sets of vessels, as, for example, that between the carotis interna and the cerebri posterior; or between branches of the same set of vessels, as, for example, that between the branches of the mesenterica superior. In each of these instances we have to do with sizable vessels and it is the absence of sizable vessels that plays an important rôle in determining what constitutes an "end artery." An example of this differentiation is found in Spalteholz's study of the distribution of the blood-vessels in muscle (14).

Ruysch (12), Haller (4), Hyrtl (6) and others, say that the anastomosing branches between the two arterial systems are of sufficient size to be seen with the unaided eye. If this be true, an injecting mass con-

taining vermilion granules should pass freely from one set of vessels to the other set of vessels, as is illustrated in the anastomoses between the superficial and deep bronchial arteries in the lung of the sheep where the anastomosing branches are discernible to the eye (Fig. 6).

In the present study, and also in my study of the bronchial artery within the lung, I have failed to demonstrate by the use of granular injecting masses any anastomoses between the bronchial and pulmonary arteries. On the other hand, by using injection masses which flow freely through the capillaries I have injected both sets of vessels but only by a backward flow of the injecting mass through the capillary network (9 e).

In my study of the bronchial artery of the pleura of the lung of man, in one instance, I inserted a cannula directly into a branch of the bronchial artery and injected an aqueous solution of Berlin blue. On cutting into the lung it was seen that the fluid had entered both the pulmonary vein and pulmonary artery. Serial sections showed that the fluid had followed the capillaries, the pulmonary artery being injected indirectly. This can in no way be interpreted as showing an anastomosis between the two arterial systems, for it is absurd to call an indirect capillary communication an anastomosis.

VASA VASORUM OF THE PLEURAL LYMPHATICS.

The presence of a network of blood-vessels about the lymphatics has been overlooked by most of the later investigators but was recognized by those of the eighteenth and early part of the nineteenth centuries. Cruikshank, for example, says: "I have injected, in quadrupeds, the arteries on the coats of the lymphatic vessels, and seen them ramifying very elegantly through their substance (1)."

Panizza (11), searching for the origin of the lymphatics, says that whenever he has seen the injection appear in the lymphatics, after having completely filled the blood-vessels, he has found a network of capillary blood-vessels of a microscopical tenuity surrounding the lymph vessels. He concludes from this that the passage of the injection is a phenomenon of porosity. Evidently, Panizza overlooked the possibilities which might result from a rupture of this network of capillaries.

Sappey (13) and Dogiel (2) seem to be the only modern investigators who have recognized the vasa vasorum of lymph vessels. Sappey says: "The lymphatics are provided with vasa vasorum which one can very easily observe under the microscope and follow into the thickness of their walls." In another place he says the wall of the lymphatic trunks is rich in vessels and nerves. Each pulmonary lymphatic, for example, being encircled by a blood vascular network with coarse longitudinal meshes.

Dogiel demonstrated a plexus of blood-vessels about the lymphatics in the ear and hind leg of the rat and in the mesentery of the rat, the cat, and the dog. In his second contribution he described a plexus about the lymphatics of the capsule of the kidney in the dog.

Arnstein, in a foot-note to the earlier of Dogiel's contributions, remarked on the fact that the blood vascular network was external to the lymphatics and supported the opinion advanced by Dogiel that they, in some way, assisted in the circulation of the lymph. In a supplementary note to Dogiel's second paper, Arnstein considers Dogiel mistaken in calling the network in the capsule of the dog's kidney perilymphatic.

When the lymphatics are collapsed they lose their beaded appearance and it is quite difficult to distinguish their valves; many run for some distance in practically a straight course, conditions which were noted by Hewson (5).

In making injections of the lymphatics of the pleura of the horse and of man it has frequently happened to me that the injection mass would fail to enter any lateral branches and, for a distance of fifty or sixty millimeters, there would be a straight trunk (Fig. 12), which eventually broke out into a complete network of lymphatics. It would be erroneous to say that there were no other lymphatics in the area through which this single trunk passed, for under a dissecting lens they could readily be made out.

When the lymphatics are collapsed there is often an appreciable distance between the encircling network of blood-vessels and the lymphatics; but, when the lymphatics are distended, the network of blood-vessels is in close contact. The former conditions are indicated in Fig. 9, the latter, in Fig. 3. Dybkowsky (3) has noted a similar relation in his study of the lymphatics of the pleura costalis.

NOTE.—After the manuscript for the above article had left the author's hand, H. M. Evans (3*) published the results of his investigations on the vasa vasorum of lymphatics. The author regrets that it appeared too late to be noted in the review of the literature, since it is the most complete work on the subject that has thus far been published.

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THE CYTOLOGICAL CHARACTERS OF THE AREAS OF LANGERHANS.

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WITH 1 PLATE.

In the course of a comparative study of the pancreas, begun in the autumn of 1905, I was struck with a peculiar reaction in certain cells of the Islets of Langerhans in the pancreas of the guinea pig—one of the first animals used in the study. This reaction,—to be described presently,—indicated the existence in the islets of two types of cells, chemically and morphologically different from each other. A part of the ensuing investigation is the subject of the present paper, which is to be followed by a further publication dealing in detail with a comparative study of the islets which I have carried on side by side with that of the islets in the guinea pig.

The principal difficulty thus far in dealing with the Islets of Langerhans has been the want of a definite method by which to distinguish the cells of the islets from the cells of the pancreas itself; for although there is an apparently constant content of islet tissue in the pancreas, and although the areas of islet tissue, in sectioned pancreas, stand out in sharp contrast with the tubules of the pancreas, the physiological distinctness of the one kind of tissue from the other is the very question upon which histologists and pathologists have most disagreed. Pancreas cells exhausted by stimulation with alkaloids, and thus thoroughly discharged of their secretion products, have thus far been indistinguishable—so far as positive evidence goes—from cells of the islets; so that it has been impossible to say that exhausted cells which are indisputably pancreas cells are not essentially the same as islet cells; and, on the other hand, that cells which are indisputably islet cells are not in reality exhausted cells of the pancreas. To establish a method of differentiation between these two orders of cells was a purpose which thrust itself forward very early in the work, as the establishment of such a method would go far toward testing the claims of the two leading theories respecting the meaning of the islets.

The adherents of one of these theories have consistently held that the islets produce a substance which, in one or another way, controls carbohydrate metabolism. This view, so carefully considered and so capably studied by Opie (14) has a particular significance when looked at in the light of my own experiments on the chemism of the islets, especially as regards the precipitability of the substances produced by the two types of cells mentioned above. What may be called the "sugar function" of the islets broadly suggests the outright physiological independence of the islets, and sharply marks off this view from that of the other party, the adherents of which have long urged the probability that the islets are merely exhausted acini which, as such, have no active function whatsoever, but are, so to speak, in a state of rest, or obscurity, and, at the end of the cycle, return to the active state as typical pancreatic acini. These being the two main interpretations of the islets, a demonstration that the cells of the islets have a chemical value of their own (and are not, as a matter of fact, merely exhausted pancreas cells, but cells which, whatever may have been their former state, have, as islet cells, a positive function) would seem to be indirectly confirmatory of the sugar theory, or confirmatory at least of the broader notion that the islets have an independent physiological activity of their own. Such confirmatory evidence, I believe, will be found in the various chemical tests described below.

A few words of history, bearing particularly on these considerations, are necessary here. For a larger historical review the reader is referred to Oppel (13) and to Sauerbeck (19). The latter has an ample review of the pathological as well as of the anatomical literature of the islets.

The structures called the Islets of Langerhans were discovered by Langerhans (10), who first called attention to them in 1869. The same year (subsequently to Langerhans's announcement) the name '*Les îlots de Langerhans*' was applied to them by Laguesse. Kühne and Lea afterwards gave them the name of "intertubular cell clumps." They have been called secondary cell groups (by Harris and Gow), *points folliculaires* (by Renault), and Islands of Langerhans (by American anatomists).

The history of the islets from the date of their discovery until 1886 is chiefly interesting for the controversies it contains, and for the opinions hazarded as to the nature and function of the structures. Langerhans himself believed them to be the end-apparatus of nerve fibers. Renault (17) described them in a very general way, and was unfortunate in being misquoted by some earlier writer who, since Renault's announcement in 1879, has been extensively followed throughout the whole of the

literature—a fact to which Sauerbeck also calls attention. Renaut has been represented as saying that the islets were lymph structures. This is not so. He did not say they were lymphoid tissue—a misconception arising from the title of his paper. He simply made a note of their existence, at the same time remarking that they had not been described before. His only reference to lymph tissue in this paragraph of his paper is to the effect that the islets (called by him *points folliculaires*) were of the size of a lymphatic follicle.

Other writers hazarded other notions without, however, coming to any satisfactory conclusion. The first definite step in that direction was taken by Lewaschew (11) who, after considerable experiment with mammals, suggested that the islets were temporarily exhausted acini which, after a period of rest, resumed the acinous form. This theory would imply a continuous transformation of acini into islets, together with a disappearance of the lumen of the acinus; and, again, a continuous transformation of islets into acini, with an accompanying rebuilding of the lumen, together with the entire complex of changes in the form of the cell, in the nucleus and its content, in the arrangement of the glomerulus of the islet capillary system, and in whatever other changes that might be necessary in this peculiar process.

Lewaschew's theory further implies that these transformations are continually going on in the entire substance of the pancreas, and he urges, in point of probability that the islet cells are in continuity with the cells of the acini. Rennie (18) has studied peculiar structures in fishes which he identifies with the islets of Langerhans, although these structures lie remote from the pancreas in the abdominal cavity. Generalizations, however, concerning the islets in other animals based upon the existence of these isolated structures in fishes, await the results of Rennie's experimental work. Lewaschew's description of transitional cells, intermediate between typical islet and the typical acinus cells is very obscure, and the obscurity is only deepened by the uncolored drawings with which his paper is illustrated. Lewaschew's views, however, have been widely accepted and still have a considerable following. Dale (?) urges them as probable from his experiments on the toad by stimulation with "secretin," although the embryological studies of Helly (6), Opie (15), and Pearce (16) seem to point the other way.

Laguesse (7) investigated the islets of Langerhans in vipers from the histological point of view, and (8) the islets in the sheep from the histogenetic point of view. His work, in these respects, has brought to the study of these structures much of the most interesting matter thus far published. Laguesse did not distinguish two types of cells, but he (as

well as others) observed the fact that the cells of the islets contained granules which could not be considered as artifacts, but were properly to be regarded as products of the metabolism of the cells themselves. He argues that the granules are not artifacts because

(a) The cells are literally crowded with brilliantly stained (safranin or gentian violet) granules.

(b) In the living structure the granules are also present when examined in serum.

(c) They are analogous (1) in their arrangement, (2) in their refraction, (3) in their brown coloration with osmic acid, and (4) in their vivid red coloration with safranin, to zymogen granules, and

(d) They are soluble in acetic acid.

Considering these facts in connection with the results of his histogenetic study of the islets in the pancreas of the sheep, he concludes that the islet and the acinous cells are transformable one into the other, as he gathers, also, from his studies of the embryo sheep alone. He believes that the islets normally furnish an internal secretion to the pancreas, but have the property of alternation from internal to external secretion, the former predominating. In the viper there are (1) secondary islets, scattered throughout the gland; developed from acini they return again to acini; and (2) permanent islets which, developed directly from the embryonic pancreatic tubes and not from acini, have no tendency to transformation into acini. But in the sheep they are atrophied, for the greater part, at a certain stage in their development, and are eliminated; in the viper they tend to persist to maturity. Laguesse finds vestiges of lumina among the cell-cords of the islets in vipers. From the above it will be seen that Laguesse coincides (with certain modifications) with the speculation of Lewaschew.

Flint (5) has studied the islets with a view of demonstrating the presence of a reticular capsule, and De Witt (3), in the course of an important experimental study of the islets, has constructed very handsome models of these structures showing their structural independence, and has furnished experimental evidence of the presence in the islets of the activator substance of Cohnheim.

The presence of granules in the islet cells was observed, as we saw above, by Laguesse; and Diamare (4) called attention to large granular, deeply staining cells in the islets of the rabbit's pancreas. W. Schulze (20) called attention to similar cells in the islets of the guinea pig's pancreas, and Mankowski (12), who repeated Schulze's work, found that on ligature of the pancreas the epithelial elements disappeared. Mankowski, however, found that an injection of silver nitrate disclosed certain

black specks in the islet cells of the guinea pig; a fact indicating the presence in the islet cells of a substance which unites with silver in a reducible form, which does not occur—so far as Mankowski's experiments show—in the pancreas cell itself. These indications, however, were not pursued farther by any of the above-mentioned observers. Negative results as to granules were found by Hanseman, Stangl, and Ssobelew, although Mankowski, however, confirmed Laguesse's observations of granules in the islets (of the guinea pig's pancreas) after fixation in Fleming's fluid. References to the publications of these observers will be found in Sauerbeck's review above mentioned.

TECHNIQUE.

Out of a rather wide range of fixing and staining fluids, three fixations and one stain were found to be the most valuable in the present investigation. The fixations used were (1) alcohol-chrome-sublimate, a fluid consisting of equal parts of a solution of potassium bichromate (3.5 per cent in water) and a saturated alcoholic (95 per cent) solution of mercuric chloride; (2) 70 per cent alcohol; and (3) Mueller's fluid with 5 per cent mercuric chloride added—a fluid called here aqueous-chrome-sublimate. Very small pieces of the pancreas (preferably from the splenic end) are taken from the living animal and quickly transferred to a generous quantity of fluid. For small pieces two hours (with one change) is sufficient in the alcohol-chrome-sublimate fluid. Tissues are left in the 70 per cent alcohol twenty-four hours. In the aqueous-chrome-sublimate from three to four hours is sufficient. It is of the utmost importance in all this technique that acetic acid be carefully avoided, as I have found that even a few drops of this acid, after repeated trials with numerous fluids containing acetic acid, were enough to vitiate the entire work. The tissues, after fixation, were hardened in the customary graded alcohols, cleared in bergamot oil, and imbedded in paraffin. Sections were cut three to five micra thick and were fixed to slides by the water method. Out of a score of stains I found the most effective to be Bensley's neutral gentian, I. To a saturated aqueous solution of gentian violet is added a saturated aqueous solution of orange G. The acid dye precipitates the basic one. This is filtered and thoroughly washed and dried. The precipitate is dissolved in 25 or 30 cc. of absolute alcohol. For staining purposes enough of this stock solution is added to 20 per cent alcohol to color the alcohol solution a deep violet.

The sections were stained 25 hours in this stain, blotted quickly and thoroughly with thick blotting paper or several sheets of filter paper in

a pad, and two methods of differentiation were used. In the first method the slide, instantly after blotting, was douched with absolute alcohol from a medicine dropper to dissolve the excess of the stain, the alcohol quickly blotted off, and the sections instantly covered with oil of cloves. The differentiation was then watched under the microscope until the zymogen granules in the acinous cells were seen to be fairly discrete, the violet stain being differentiated out of the cytoplasm which, with this method, retains the brownish-yellow of the orange G. In the second method the sections were quickly blotted, as before, and the differentiation done with acetone (dimethylketone). The sections were douched with acetone from a medicine dropper, quickly placed under the microscope, and when the zymogen granules appeared, as before, the slide was placed in xylol. Xylol was also used for the final clearing of the alcohol-differentiated sections. The sections were then mounted in Canada balsam.

CHEMICAL CHARACTERS.

The first sections examined were those fixed in alcohol-chrome-sublimate. This fixation is an admirable precipitant of the zymogen granules in the pancreas cells, but it has the disadvantage of shrinking the tissues somewhat. The granules of zymogen in the acinous cells are discrete and handsomely stained by the dye. The islet cells are somewhat shrunken, the majority of them taking up the yellow of the orange G. In the center of the islet, sometimes eccentrically placed, and seldom near the edges, were seen a number of conspicuous and brilliantly violet cells, apparently much larger than the remaining cells of the islet and most frequently seen in a sharply defined group (Fig. 1). They seldom appear scattered or isolated. Examined with powers which distinctly show the individuality of the zymogen granules, the large violet cells of the islet appear to be of a diffuse color; but when examined under 2 mm. apochr. these cells are found to be filled with granules very much smaller than the zymogen granule of the pancreas, but quite distinct none the less. The nuclei are large and vesicular, and at times surrounded by a very narrow clear zone in which there is seen occasionally a centrosome. The remaining cells of the islet, considerably more numerous than the cells reacting to the violet stain, show no granules in the cytoplasm, are smaller than the granular cells, and present other morphological characters which differentiate them from the latter. I will recur to these matters presently.

Preparations Fixed in 70 Per Cent Alcohol.—The presence of granules in certain of the islet cells, simultaneous with the presence of similarly reacting granules of zymogen in the acinous cells, suggested the query

whether or not the chromatophile granules of the islet cells were zymogenic; whether or not the use of a reagent which would be a solvent for zymogen would nevertheless act as a precipitant for the granular substance in the islet cells. This experiment was made with alcohol of 70 per cent strength. Small pieces of pancreas of the guinea pig were fixed, therefore, in 70 per cent alcohol and stained with neutral gentian. In sections treated in this way the acinous cells were quite devoid of zymogen granules except at the extreme edge of the piece, where a partial fixation of the granules was obtained, whereas the islets presented the same appearance as in the sections fixed in the alcohol-chrome-sublimate. The same groups of violet-granulated cells were present. But, as the alcohol had not dissolved out the prozymogen of the acinous cell, the query still remained whether or not the substance in the islet cell granule partook of the nature of prozymogen. To check this query I applied MacCallum's iron reaction on these sections and failed to bring out the slightest trace of Prussian blue in the suspected cells of the islets, except in the nuclear chromatin.

Preparations Fixed in Aqueous-Chrome-Sublimate.—The use of this fluid I found advisable after exhausting the list of desirable acetic fixations and reducing the quantity of acetic acid to an almost negligible proportion. Sections from tissues fixed with an acetic mixture were invariably blank as to granules in the cells of the islets. But with tissues fixed in aqueous-chrome-sublimate a most unlooked-for result appeared. The large cells of the islets which had been filled with violet granules in sections fixed with the other fluids were quite free from stained granules in the sections fixed with aqueous-chrome-sublimate, whereas, on the contrary, the cells which gave no violet reaction with the other fluids, in this one were filled with granules of a brilliant violet, while now it was the small groups of large cells that were colored with the yellow-brown of the orange G (Fig. 2).

From a consideration of these facts several conclusions arise. These conclusions have to do with the microchemistry of the cells of the Islets of Langerhans in the guinea pig's pancreas, and they may be stated somewhat as follows:

1. The Islets of Langerhans in the pancreas of the guinea pig consist of two types of cells: (a) a type containing a granular substance that is precipitated by alcohol of a strength of from 50 to 70 per cent; and, (b) a type, the granular content of which is precipitated by an aqueous-chrome-sublimate fluid of the general character described.

2. The granular substance that is precipitated by alcohol is dissolved

by the chrome-sublimate fluid, and the substance that is precipitated by the chrome-sublimate fluid is dissolved by alcohol.

3. Neither of these granular substances is of the same chemical character as the zymogen granules of the pancreas cell, and

4. Neither of them is of the same chemical character as the prozymogen of the pancreas cell.

To avoid cumbersome periphrase and repetition I will hereafter designate the cells in which the granules are fixed with alcohol as A cells, and these in which the granules are fixed with the chrome-sublimate fluid as β cells.

That the chemical nature of the granules in the A cells differs from that of the granular content of the β cells is borne out by the difference of the reaction of these substances to various fixing reagents non-alcoholic in character, and for the most part very simple in composition. The results follow:

Saturated Aqueous Sublimate.—With this fixation the islet cells act in virtually the same manner as with aqueous-chrome-sublimate, with the exception that the tissues are rather shrunken than the reverse. The A cells remain devoid of basic granules, the β cells are very well preserved, and, throughout the whole of their cytoplasm, they are crowded with the violet granules, which are, however, not as brilliantly stained as in preparations fixed with aqueous-chrome-sublimate. The zymogen granules in the acini, on the contrary, are well fixed and react with a brilliant stain to the neutral gentian.

Saturated Picric Acid.—This fixation is poor, in a general way, and both types of islet cells are entirely devoid of granules, taking up only the plasma stain. The zymogen granules, here also, are sharply defined and stain well.

Nitric Acid, 10 per cent.—The general fixation is poor, but the acid seeks the A cells much in the same way as does alcohol, and the granules in them are well preserved. The β cells, with this fixation, remain clear of granules altogether. So far as the A cells are concerned, the picture here presented is substantially the same as that obtained with the use of alcohol-chrome-sublimate and 70 per cent alcohol.

Formol, 10 per cent.—This fluid fixes the granules in the A cells, leaving the β cells clear. Although the stain is by no means as brilliant as that obtained with the three principal fluids, yet the individuality of the granules in the A cells is capitally preserved. The zymogen granules in the pancreas cells are broken down and diffused.

Chromic Acid, 1 per cent.—In this fixation the granules in the A and

β cells both remain unfixed, and the islet appears uniformly brownish yellow. The zymogen granules, on the contrary, appear to be well fixed and react readily to the neutral gentian.

Aqueous-Chrome-Sublimate plus 5 per cent of 10 per cent Nitric Acid.—This fixation gives much the same result as that with aqueous-chrome-sublimate, but the chromatic effects are not so clear. The β cells are admirably picked out and almost invariably show the entire cytoplasm crowded with granules. In this fluid the zymogen granules are well preserved and take the stain fairly well.

The affinity of the two types of cells for certain fixing agents is peculiarly brought out in tissues fixed in a *combination of these fluids*. Thus in tissues fixed in chrome-sublimate solutions with equal parts of alcohol added, an islet here and there near the edge of the section shows both types of cells equally granulated; and the same is true of islets near the edge of sections from tissue fixed in aqueous-chrome-sublimate to which has been added 5 per cent of 10 per cent nitric acid.

These various tests, together with those furnished by the use of the three principal fluids above mentioned, enable us to make certain positive statements concerning the nature of the two substances contained in the A and β types of cells in the guinea pig's pancreas. That contained in the A cell is fixed by alcohol, by a 10 per cent solution of nitric acid, and by a 10 per cent solution of formol. In all these fixations it is stainable with Bensley's neutral gentian. It is soluble in acetic acid, in saturated aqueous mercuric chloride, in non-alcoholic chrome-sublimate fluids, in these last-mentioned fluids also in the presence of nitric acid, in saturated aqueous picric acid, and in 1 per cent solution of chromic acid. The substance is chemically different from that in the zymogen granules, for in all these solutions the zymogen granule is fixed and remains stainable with neutral gentian, while on the contrary the zymogen granule is soluble in alcohol in which the A granules are well preserved.

The substance in the granule of the β cell is fixed in aqueous saturated solution of mercuric chloride, in chrome-sublimate fluids in the presence of nitric acid, and in non-alcoholic chrome-sublimate fluids. It is soluble in alcoholic solutions, in acetic acid, in aqueous saturated solution of picric acid, in 10 per cent solution of formol, and in 1 per cent solution of chromic acid. And it differs chemically from the zymogen granule of the pancreas because the latter is uniformly fixed by the above solutions with the exception of formol, in which it is not completely dissolved but only partially preserved.

These considerations go to show in contrast to Laguesse's theory that

the substances contained in the granules of the A and of the β cells are chemically different from the substance in the zymogen granule; and are different chemically from each other.

MORPHOLOGICAL CHARACTERS.

Coincidental with these chemical differences are found certain differences in the morphological characters of the A and β cells of the islets in the guinea pig's pancreas. The A cell is comparatively large and its nucleus is, for the most part, elliptical, although frequently it is circular (Fig. 1). It is markedly vesicular, strikingly large and vivid, and its chromatin content is very small. The chromatin is distributed in a few small, spherical masses, and this contributes, in section, to the lucid, vacuous, and prominent appearance of the nucleus of these cells. In some of the cells the granules are packed together throughout the entire cytoplasm and seem to lie directly against the nuclear membrane. In others the granules are determined in a mass bordering closely on the capillary, while the remainder of the cytoplasm is comparatively or completely free of them. The cells are polygonal and stand out in high relief against the lighter and yellowish background formed by the mosaic of the β cells. This is true only when the stain used is the neutral gentian of Bensley. The A cells can be chromatically distinguished only with great difficulty when other stains are employed—a fact struck out after trying a score or so of different basic dyes. Gentian violet, safranin, licht-grün, and other granule stains gave negative chromatic results, although the α cells could be recognized by their conspicuous size even when the section was treated only with a plasma stain.

The β type of cell appears, as a rule, considerably smaller and is, at the same time, vastly more numerous in the islet. Entire cords of them, uninterrupted by the presence of the A cells, appear in the picture, and almost invariably the cytoplasm of the entire cell is packed with the violet granules, which are uniformly distributed around the nucleus and which everywhere border on the capillaries. The nucleus of the β cell is invariably centrally placed, is smaller than the nucleus of the A cell, circular, markedly less vesicular than the nucleus of the A cell, and is also distinguished from the nucleus of the A cell by the comparatively large quantity of chromatin it contains. In the nucleus of the β cell the chromatin is frequently seen in the form of fine strands forming a network. In some of the islet cells there were found, indifferently as to either kind, a centrosome and, now and then, a mitotic figure. The cytoplasm consists of a delicate network.

SUMMARY AND CONCLUSIONS.

Recapitulating the facts above described the following positive statements may be made:

1. In the Islets of Langerhans in the guinea pig's pancreas two types of cells, morphologically and physiologically distinct, are demonstrable. These cells show constant reactions to constant chemical tests. I have called these cells A and β cells, respectively.

2. The granular content of the A cell differs chemically from that of the β cell.

3. The granular contents of the A cell and of the β cell, while differing chemically from each other, differ chemically from the granular content of the pancreas cell, and cannot, therefore, be identical with zymogen.

4. The granular contents of the A and of the β cell differ chemically from the prozymogen manufactured by the pancreas cell as the antecedent of the zymogen granule of the pancreas cell.

5. The chemical and morphological differences between the A and the β cell are correlated; that is, the relations between the anatomical and physiological characters of both types are found to be constant.

In drawing conclusions from these facts one is led to the conviction that the Islets of Langerhans are structures which in all probability have the function of producing a twofold substance which, poured into the blood stream, has an important effect upon metabolism. That this dual character of cell in the islet is constant throughout the entire class of mammals, if not throughout the entire phylum of vertebrates, is indicated as probable from the results of the comparative study now in progress, which I hope to make the subject of a future publication. The prospects seem to point to certain peculiar variations in the character of these cells in herbivora and carnivora, and to striking and highly suggestive variations among herbivora themselves.

While these results do not prove that pancreatic cells do not transform into islet cells, or vice versa, they furnish very strong reasons for holding that under normal conditions the islets are physiologically independent of the rest of the pancreas—a conclusion in accord with the observations of De Witt and Flint as to the framework and architecture of these structures, and with those of Opie, Pearce, and Helly as to the early differentiation in the embryo of the specific cells which are their histogenetic source.

It is but rational to conclude from the chemical evidence that the substances produced by the two types of cells of the islets are not to be classified with zymogen. If the cells of the pancreas have the power of

transforming themselves into the cells of the islets which I consider improbable, that transformation must be regarded as a physiological as well as a morphological one. In the course of an examination of many hundreds of islets in the pancreas of the guinea pig I have been able to find but one example of what might be interpreted as a vestige of a lumen; and this singular structure seemed to be formed by cells of the A type.

There is one remaining aspect of the problem to be touched upon before closing. This is the possibility that the A and the β types of cell are in reality two different phases of the same cell—a notion by no means improbable even in the face of the chemical evidence to the contrary. Professor Bensley, who has examined my preparations with considerable care, has pointed out cells which seem (from the anatomical side) to be intermediate between the two types, especially in certain preparations which, at his suggestion, were treated with Ehrlich's hæmatoxylin before they were submitted to the neutral gentian bath. But even granting the truth of this observation, the force of the general argument remains. If the A and β cells are really phases of one and the same cellular structure, their different chemical characters suggest that they are engaged in the manufacture of two different secretions. If we conceive that the A cell changes into the β cell, or vice versa, we must conclude that the change implies the taking on of a different physiological activity. Whether or not these two different secretions have to do with the pancreas itself or, through the pancreas with functions lying, in their special or general effects upon the chemistry of the body, near to or remote from, the pancreas, is a matter to be determined by further research.

I have to thank Professor Bensley, who was kind enough to direct my researches, for his lively interest in the work, for his invaluable suggestions as to technique, for his assistance in the interpretation of difficult *nodi* that arose as the work developed, and for having made preliminary reports of the work to the Association of American Anatomists. My thanks are also due to Mr. Leonard H. Wilder for the fidelity of the drawings which accompany this paper.

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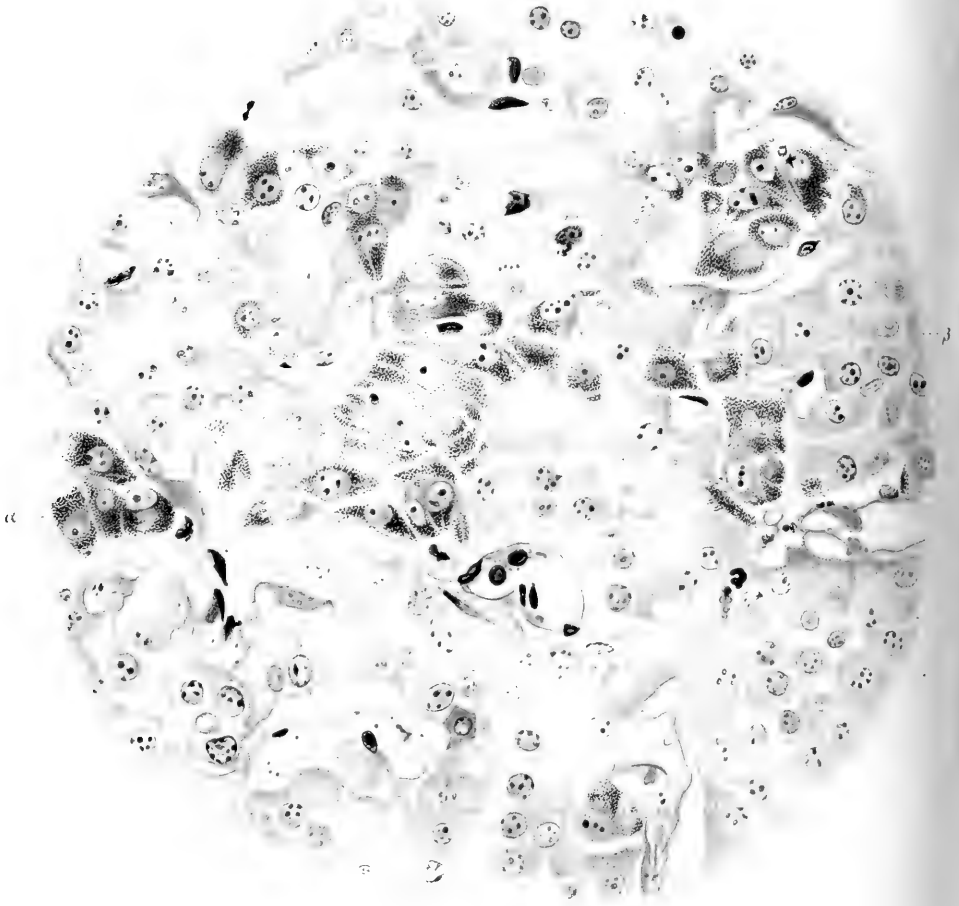
EXPLANATION OF FIGURES ON PLATE I.

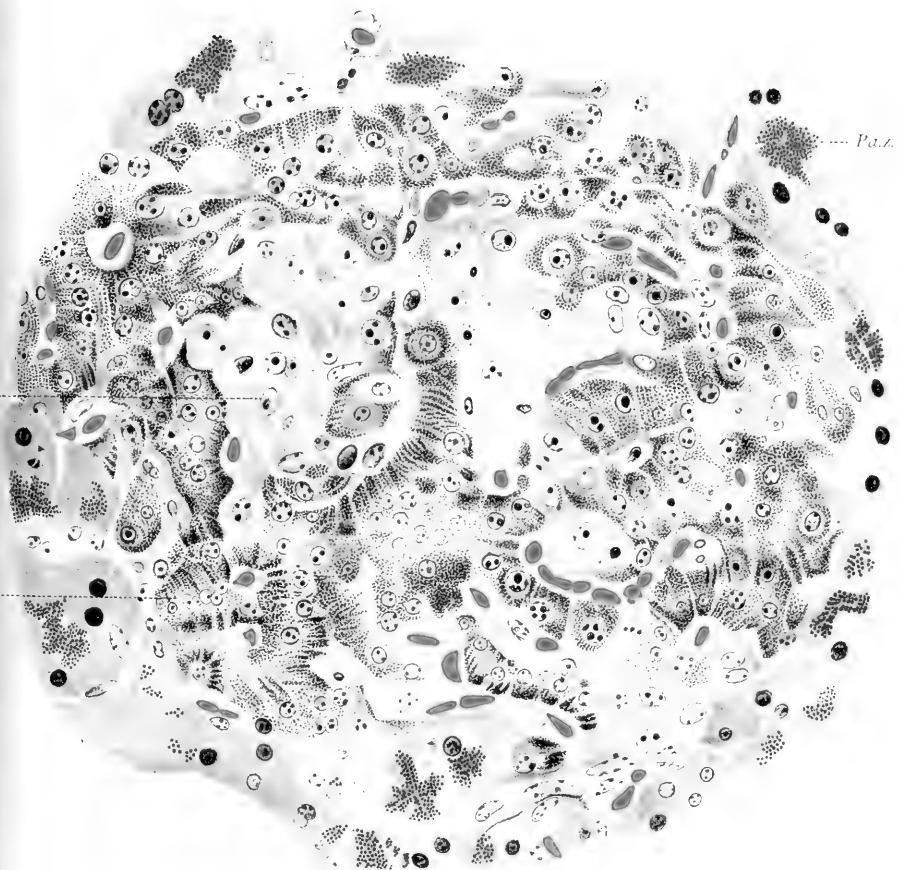
FIG. 1. Section of an Islet of Langerhans of the Guinea pig, fixed in 70 per cent alcohol, stained with Bensley's Neutral Gentian, showing the A cells of the islet filled with intensely stained granules, β cells orange. Zeiss apo. 2 mm., oc. 8.

FIG. 2. Islet of Langerhans of the Guinea pig; aqueous chrome-sublimate fixation; neutral gentian stain; showing β cells filled with minute violet granules; A cells orange, the staining reaction being reversed. Pancreatic acini, Pa. z. with zymogen granules are seen at the edges of the section.

THE CYTOLOGY OF THE AREAS OF LANGERHANS

M.A. LANE





STUDIES ON THE VARIATION AND CORRELATION OF
SKULL MEASUREMENTS IN BOTH SEXES OF MATURE
ALBINO RATS (*MUS NORVEGICUS* VAR. *ALBUS*).

BY

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WITH 1 FIGURE AND 10 TABLES.

The present investigation was undertaken to determine the size of the skull and the relative development of its constituent parts in the adult albino rat. To this end the biometric method was employed with the idea that in this way it would be possible to obtain more precise results and also with the idea of later comparing these skulls with those of the hybrids between *Mus norvegicus* and *Mus rattus*.

MATERIALS AND METHODS OF MEASUREMENT.

For the present study 53 male and 51 female skulls of mature rats (rats more than 150 days old) were measured. The following measurements were made with vernier calipers: (1) the length of the entire skull; (2) the fronto-occipital length; (3) the zygomatic width; (4) the length of the nasal bone; (5) the height of the skull; (6) the width of the cranium or the squamosal distance. In every case the maximum length alone was recorded.

In the present paper the horizontal straight line joining the tip of the nasal bone to the end of the occipital bone is called the length of the entire skull. This however is not exactly equal to the sum of the length of the nasal bone and that of the fronto-occipital.

The fronto-occipital length was determined in the following way: Since the length measured with the calipers from the tip of the nose to the posterior end of the inter-parietal bone is not always equal to the length measured from the tip of the nose to the end of the occipital bone, both measurements were taken (See Fig. 1). The latter measurement is usually the longer. The difference between the two measurements was added to the length from the tip of the frontal bone to the end of the inter-parietal bone, and the sum was called the fronto-occipital length.

The width of the cranium (squamosal distance) was determined by taking the maximum distance between the two points (right and left) where the zygomatic bones rest on the lateral walls of the cranium. The height of the skull was determined by measuring a perpendicular distance between the greatest convexity of the parietal bone in the median line and the junction line between the basi-occipital and the basi-sphenoidal bones on the ventral surface.

The cranial capacity was determined in the following way: The skull was held vertically, with the nose downwards and was filled with fine shot (No. 11) to the upper level of foramen magnum and then the nose of the skull gently struck twice against the palm of the hand. The space

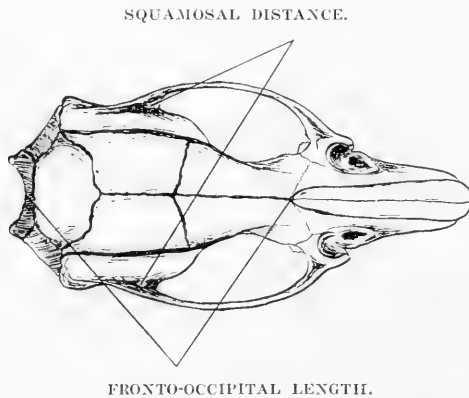


FIG. 1. Diagram of the skull of the adult albino rat, seen from above.

thus formed was again filled. Although this is a simple procedure yet it needs the greatest care and much practice in order to produce uniform results. The slightest variation will easily cause differences of more than one gram in the weight of the shot. The greater the experience of the observer the more uniform are the results. By practice the author has been able to reduce the difference between the first and second filling to less than one decigram in eight cases out of ten. The distribution of the errors in this work was found to follow the Gaussian normal curve and therefore it is inferred that the number of minus errors is the same as the number of plus errors. As a matter of fact the average difference between the first and second fillings did not exceed one per cent. The cranial capacity thus determined was finally transformed into cubic centimeters of brain substance (see page 435).

TABLE I.

	Mean.		Standard deviation.		Coefficient of variation.		No. of rats.
		Difference.		Difference.		Difference.	
Length of the entire skull.	♂	43.255 ± 0.166	1.706 ± 0.204	1.786 ± 0.117	0.530 ± 0.144	4.129 ± 0.271	53
	♀	41.549 ± 0.119	3.944 %	1.256 ± 0.084		3.016 ± 0.202	51
Zygomatic width.	♂	21.745 ± 0.109	0.820 ± 0.137	1.177 ± 0.077	0.301 ± 0.064	5.412 ± 0.356	53
	♀	20.925 ± 0.083	3.771 %	0.876 ± 0.059		4.186 ± 0.280	51
Length of the nasal bone.	♂	16.958 ± 0.096	1.266 ± 0.122	1.038 ± 0.068	0.245 ± 0.086	6.121 ± 0.403	53
	♀	15.693 ± 0.075	7.465 %	0.793 ± 0.053		5.053 ± 0.338	51
Fronto-occipital length.	♂	27.264 ± 0.093	0.911 ± 0.126	1.007 ± 0.066	0.103 ± 0.090	3.693 ± 0.242	53
	♀	26.373 ± 0.085	3.341 %	0.904 ± 0.060		3.427 ± 0.229	51
Squamosal distance.	♂	15.273 ± 0.010	0.217 ± 0.040	0.388 ± 0.022	—	2.213 ± 0.145	53
	♀	15.056 ± 0.039	1.420 %	0.409 ± 0.027	—	2.716 ± 0.181	51
Height of skull.	♂	11.493 ± 0.049	0.354 ± 0.065	0.526 ± 0.034	0.151 ± 0.043	4.576 ± 0.300	53
	♀	11.139 ± 0.035	3.080 %	0.375 ± 0.025		3.366 ± 0.225	51
Cranial capacity.	♂	10.896 ± 0.068	0.528 ± 0.098	0.735 ± 0.048	—	6.745 ± 0.444	53
	♀	10.368 ± 0.070	4.845 %	0.743 ± 0.050	—	7.166 ± 0.481	51
Body-weight.	♂	214.886 ± 5.318	47.541 ± 5.982	52.887 ± 3.760	32.413 ± 4.088	25.076 ± 2.675	45
	♀	167.345 ± 2.739	22.170 %	20.474 ± 1.605		12.235 ± 0.974	37
(Length × width × height) ¹ .	♂	16.875 ± 0.044	0.452 ± 0.056	0.478 ± 0.031	0.096 ± 0.040	2.832 ± 0.186	53
	♀	16.423 ± 0.036	2.678 %	0.382 ± 0.026		2.326 ± 0.155	51

DETERMINATION OF THE MEANS AND VARIABILITY OF THE SEVERAL MEASUREMENTS.

In Table I are exhibited the means of the several measurements, the standard deviations, the coefficients of variation and the differences between the two sexes, with their respective probable errors. These values were determined by the usual biometric formulæ (Davenport, 04). As one would expect, the mean values in the males are always higher than in the females. Since in the present investigation the total number of measurements of both sexes was not large, it is important to compare the differences between the sexes with the corresponding probable errors in order to see whether or not the differences here found are to be considered significant. Table I shows clearly that in all cases the differences are greater than three times the probable errors. The maximum difference occurs in the nasal bone; ten times the probable error, and the minimum occurs in the width of the cranium, five times the probable error. This indicates that the characters under consideration are really greater in the male. The maximum percentage differences occurs in the nasal bone (7.5 per cent) and minimum in the width of the cranium (1.4 per cent) while the remaining differences are nearly similar (3.1 per cent to 3.9 per cent).

From the percentage differences here found it would seem probable that if we compared the mature male and female skulls having *the same total length*, the length of the nasal bone in the male would be longer than that in the female, and since the length of the entire skull depends on the combined length of the nasal bone and the fronto-occipital length, it would follow that the fronto-occipital length or the length of the cranium, would be less in the male than in the female. In order to test this conclusion in any individual case it is necessary to determine whether or not the total length of the skull and of the nasal bone on one hand, and the total length of the skull and fronto-occipital length on the other hand, are closely correlated. This point will be more fully discussed after the coefficients of correlation have been determined.

(a) *Range of variates and rate of increase of the various characters associated with changes in the size of the entire skull.*—The two extremes of the various characters, as well as the rate of increase of those characters associated with the increase in the length of the entire skull, is somewhat different according to sex as is shown in Table II.

As is shown in Table II, the absolute range between the two extremes is always slightly greater in male than in female except in the case of the

width of the cranium or squamosal distance and in the fronto-occipital length where they are nearly alike in the two sexes. Generally speaking the changes associated with the increase in the length of the entire skull are relatively greater in female than in male. This is especially evident in the length and width of the cranium and the zygomatic width, although the absolute amount of change is considerably less in the female than in the male. In the female the relative change is very slightly greater in the nasal bone and is the same in the height of the skull. The same table

TABLE II.

	Male.			Female.		
	Minimum mm.	Mean* mm.	Maximum mm.	Maximum mm.	Mean* mm.	Minimum mm.
Length of the entire skull.	39.4	43.3	47.4	44.5	41.5	38.9
Rate	100	100	100.	100.	100.	100.
Zygomatic width.	19.6	21.7	24.8	23.4	20.9	18.9
Rate	49.8	50.2	52.3	52.5	50.3	48.5
Length of the nasal bone.	14.7	17.0	18.7	17.8	15.7	14.4
Rate	37.3	39.2	39.3	40.0	37.7	37.0
Fronto-occipital length.	24.9	27.3	28.8	28.2	26.4	24.9
Rate	63.2	63.0	60.7	63.3	63.5	64.0
Squamosal distance.	14.6	15.3	16.2	16.2	15.1	14.4
Rate	37.0	35.3	34.1	36.4	36.2	37.0
Height of Skull.	10.4	11.5	13.0	12.2	11.1	10.3
Rate	26.4	26.5	27.4	27.4	26.8	26.4

shows us clearly that in respect to the length of the entire skull, the width and length of the cranium are greater in female than in male although in both sexes the absolute amount of change is less than for any other characters measured. The relatively slight increase in the width of the skull (zygomatic width) associated with the increase in the length of the entire skull, has also been noticed by Allen, 94, in the case of *Neotoma micropus*.

When the mean values (Table II) are treated in the same manner as the two extremes, additional light is thrown on the changes following the increase in the length of the entire skull. In all characters, except the

* Taken from Table I.

length of the nasal bone, the female gives relatively greater values than the male. Although the excess shown in the female is not large in the case of the zymatic width, and in the case of the height and length of the cranium yet the width of the cranium or squamosal distance is decidedly greater in the female than in the male, as has already been seen from the measurements of the extremes. When mean values for the length of the entire skull are reduced to the same standard and the associated measurements are compared, all the three diameters of the cranium of the female are seen to be relatively greater than those of the male in respect to the length of the entire skull. The cube root of product of these three diameters in the case of the male is 39.01 per cent of the length of the entire skull and in the case of the female 39.52 per cent, thus indicating that we might expect the relative capacity of the female cranium would be sensibly greater than that of the male. This apparent superiority of the female cranium over that of the male is not due, however, to the relatively greater lengths of the three cranial measurements of the female, but is due to the fact that the nasal bone in the male skull is considerably longer, thus producing a somewhat less percentage value for cube root of the product of the three diameters of the male cranium. As a matter of fact, when the length of the entire skull is equated either to male or to female standard by means of the characteristic equations and the resulting measurements of the cranium in the two sexes are compared, the size of the cranium in the two sexes is almost identical. We shall discuss this point later (page 436). It is therefore enough at the present moment to see that the somewhat greater percentage values obtained from the three diameters of the cranium, when mean values for the length of the entire skull are reduced to 100, indicate that the nasal bone is much shorter in the female, and vice versa.

(b) *Relative variability in the two sexes.*—The relative variability in the two sexes is a question which has passed through various phases during the last century. The several opinions held by different investigators are fully summarized by Havelock Ellis in his book on "Man and Woman," 94. The history of this question may therefore be omitted. It is, however, important to note here that the quantitative investigation of this question has been made for the most part on the human subject.

As is shown in Table I, the standard deviation is in every case greater in the male than in the female except in the case of the squamosal distance. Since the standard deviation measures the amount of concentration of the variates about the mean, the greater the standard deviation the less will be the concentration and consequently the greater will be

the variation in the character under consideration. Therefore the greater values obtained for the male means simply that the characters in question vary more in the male than in the female.

The absolute value of the standard deviation is widely different throughout the table. This is due to the fact that in these cases the standard deviation is a concrete number and therefore the variabilities can not be directly compared with one another since the magnitude of the characters as well as the unit taken for grouping is never the same. The coefficient of variation, however, enables us to compare the relative amount of variability of the characters measured in different units since it is one hundred times the quotient of the standard deviation divided by the means. From Table I it was found that the values of the coefficients of variation in the male are always greater than that in the female, except in the case of the squamosal distance in which the reverse is true.¹ Here also the length of the nasal bone shows the greatest variation and the zygomatic width comes next while the least variation is found in the width of the cranium. Brewster, 97, also noticed a greater variation in both the length of the nasal bones and zygomatic width than in any other measurements made on the different parts of the skull. His studies were made on the lynx (*Lynx canadensis*); cat (*Felis domesticus*); and red fox (*Vulpes fulvis*). The methods employed by Brewster for determining his coefficients of variation are so different from those used in the present investigation that two sets of figures can not be directly compared. Except the length of the cranium, the remaining characters tend to show the existence of a sexual difference as to the relative variability, that is the male tends to vary more than in the female.

The mean values obtained from the cranial capacity, body-weight, and cube root of the product of the height, length and width of the cranium are also greater in the male than in the female. The standard deviations as well as the coefficients of variation indicates a relation similar to that found in the skull measurements, that is, the male tends to show in these characters a greater variability than the female.

(c) *Coefficients of variation in man and rat.*—The following table was compiled in order to show the variation in the human skull as compared with that for the skull of the albino rat:

¹ Slightly greater variability in the female is also found in the cranial capacity, nevertheless the result is insignificant owing to the greater probable errors in this case.

TABLE III.

SKULL CAPACITY.

	Male.	Female.	Investigator.
Etruscan	9.58	8.54	Pearson and Lee.
Modern Italian.....	8.34	8.99	Pearson and Lee.
English	8.28	8.68	Macdonell.
Egyptian mummies.....	8.13	8.29	Pearson and Lee.
Modern German.....	7.74	8.19	Pearson and Lee.
Naquada	7.72	6.92	Fawcett.
Parisian French	7.36	7.10	Pearson and Lee.
Aino	7.07	6.90	Pearson and Lee.
Albino rat	6.75	7.17	Hatai.

SKULL HEIGHT.

English	4.21	3.96	Macdonell.
Aino	3.67	3.18	Pearson and Lee.
German	4.47	3.91	Pearson and Lee.
Albino rat	4.58	3.37	Hatai.

SKULL LENGTH.

English	3.31	3.45	Macdonell.
Naquada	3.09	2.96	Fawcett.
Aino	3.20	3.08	Pearson and Lee.
German	3.37	3.57	Pearson and Lee.
English (base of skull)...	4.07	4.11	Macdonell.
Albino rat	3.69	3.43	Hatai.

SKULL BREADTH.

English	3.75	3.54	Macdonell.
Naquada	3.42	3.42	Fawcett.
Aino	2.76	2.68	Pearson and Lee.
German	3.89	3.39	Pearson and Lee.
Albino rat	2.21	2.72	Hatai.

As will be seen from Table III, so far as the cranial measurements are concerned the coefficients of variation in the albino rat are slightly less than in man, though the difference is by no means large. It is interesting to note that the magnitude of variability both in man and rat is in the same order for corresponding characters, that is the variability of the cranial capacity is considerably greater than the remaining three linear measurements both in man and rat. This is perhaps due to the fact that the cranial capacity is itself highly variable and in addition the technical difficulties of determination of the capacity influence the results further.

Pearson, 97, 01, with reference to variation in the several dimensions of the human skull, thinks that with advance in civilization woman tends to gain in variability on man (see on Aino and Naquada races). Nevertheless, if we examine the data recently obtained by Fawcett, 02,

and Macdonell, 02, Pearson's conclusion, so far as the measurements of the skulls are concerned, is not well supported. For instance the total averages of the coefficients of variation in man (Aino and Naquada races are excluded) are 4.99 per cent while in woman it is 4.84 per cent. Even if we include Aino and Naquada, the relative variability is still in favor of man. The same is true also for the albino rat (4.31 per cent male, and 4.17 per cent female). Therefore so far as the data at hand are concerned the several measurements of the male skulls show a general trend to a greater variability than those of the female. Since in all cases, the number of the skulls examined is not large, it is evident that this point needs still further study.

DETERMINATION OF THE COEFFICIENTS OF CORRELATION.

The degree of correlation between any two characters is usually determined by the formula: $Y = \frac{\varepsilon(x, y)}{n \cdot \sigma_1 \sigma_2}$, where x, y are deviations from the means of the two correlated characters and δ_1, δ_2 the respective standard deviations. It will be advantageous to discuss the coefficients of correlation under five headings.

(a) *Correlation between the length of the entire skull and the other cranial measurements.*

TABLE IV.

	Male.	Female.	Difference.
Length of entire skull and zygomatic width948 ± .011	.836 ± .029	.113 ± .031
Length of entire skull and fronto-occipital length946 ± .010	.956 ± .008	— .010 ± .013
Length of entire skull and length of nasal bone845 ± .027	.890 ± .020	.044 ± .034
Length of entire skull and squamosal distance582 ± .061	.309 ± .085	.273 ± .105
Length of entire skull and height of skull555 ± .064	.314 ± .085	.241 ± .107

As one would expect, the degree of correlation in the first three cases is very high in both sexes. Since the length of the skull depends on the length of the nasal bone and the fronto-occipital length, any change in the length in the entire skull must involve a change in the length of either the nasal bone or the fronto-occipital length or in both. The correlation shows, however, that change in the length of the skull is associated with changes in both the nasal bone and the fronto-occipital length; the latter

change being the better correlated. The high correlation between the length of the skull and the zygomatic width means a regular enlargement in the transverse diameter associated with a change in length. The correlation between the length of the entire skull and width (squamosal distance) and height is comparatively low in both sexes. Therefore it is concluded that change in both width and height of the cranium corresponding to the change in the length of the entire skull is less regular than in the other three characters already discussed. It is of interest to note that although the correlations between the length of the entire skull and the width and height of the cranium are not high, yet the correlation between the width and height of the cranium is high, especially in the male. (See Table V.)

(b) *Correlation between the height, length, and width of the cranium.*
—Table V shows the coefficients of correlation between the three linear measurements just mentioned.

TABLE V.

	Male.	Female.	Difference.
Fronto-occipital length and squamosal distance665 ± .052	.397 ± .080	.268 ± .095
Fronto-occipital length and height of skull571 ± .063	.387 ± .080	.184 ± .102
Squamosal distance and height of skull707 ± .046	.425 ± .077	.282 ± .090

As the table indicates the width (squamosal distance) and height of the cranium give the highest correlation in both male and female, the length and width come next, while length and height give the lowest correlation. Although in every case the male sex gives the higher correlation, the comparison between the differences and probable errors shows that we can not lay much weight on this apparent superiority, except in the case of the height and width, since in the two other cases the differences are smaller than three times the probable error. For comparison there are no data available except the observation by Pearson and Lee, *or*, on the human skull, their results are given in Table VI.

TABLE VI.

		Male.	Female.
Length and breadth	German286 ± .062	.488 ± .052
	Aino432 ± .059	.377 ± .073
Length and height	German	-.098 ± .067	.314 ± .061
	Aino501 ± .054	.349 ± .075
Breadth and height	German071 ± .067	.276 ± .063
	Aino345 ± .064	.178 ± .082

Pearson and Lee's data show that the correlation in the first two instances (length and breadth, and length and height) are about the same and are much higher than that of the last case (breadth and height). In the albino rat, length and width, and length and height also give nearly the same degree of correlation as in man, but the values obtained are smaller than for the width and height. Thus in this respect the rat and man show reverse relation. The difference is probably due to differences in relative development of the several bones of the cranium depending on the skull form characteristic for the two species.

Whether or not it is a general phenomenon in the lower mammals that the coefficient of correlation is higher in the male than in the female, as is shown in the albino rat, needs further observation. It has been pointed out by Pearson, 97, that in the human race with advancing civilization woman tends to gain in correlation on man. It is clearly seen from the data given above that in German skulls the coefficients of correlation tend to be higher in the female than in the male while in the Ainos the reverse relation is true, favoring the view maintained by Pearson. Pearl, 06, who compared a large number of brain records with other body characters in the case of Bohemians, Bavarians, Hessians, and Swedes found that the weight of the brain also tends to be more highly correlated with other characters in the female than in the male.

(c) *Correlation between cranial capacity and other cranial measurements.*—The cranial capacity as determined with the shot gives high degree of correlation with the length of the entire skull as well as with the three diameters of the cranium. This is shown in the following table:

TABLE VII.

	Male.	Female.	Difference.
Cranial capacity and length of the entire skull678 ± .050	.484 ± .072	.194 ± .088
Cranial capacity and fronto-occipital length761 ± .039	.577 ± .062	.184 ± .073
Cranial capacity and squamosal distance838 ± .028	.632 ± .057	.206 ± .063
Cranial capacity and height of skull	.760 ± .039	.666 ± .053	.094 ± .066
Cranial capacity and (height × length × width) ¹ / ₂836 ± .028	.854 ± .026	—0.018 ± .038

As is shown in Table VII the coefficients of correlation are in general higher in the male than in the female, but when compared with their respective probable errors the differences are not large enough to warrant laying much stress on the apparent superiority in male sex, except in the one case of the cranial capacity and squamosal distance. It is therefore

safe to say that according to this test the degree of correlation is nearly the same in both sexes. It is interesting to note that the cranial capacity is best correlated with the width (male and female) and the height (male) of the cranium. Therefore a prediction of brain weight based on the cranial measurements would give least error when based on the width. The length of the entire skull gives the lowest degree of correlation in both sexes. This is of course what one would expect since the length of the entire skull is least correlated with both the height and width of the cranium, especially in the case of the female. The product of the height, length, and width of the cranium is highly correlated with the cranial capacity and the correlation, although a trifle higher in the female, is nearly the same in the two sexes. This is also an anticipated result since roughly speaking the capacity should be closely related to the product of three diameters. In this case one might expect to find the correlation almost unity, but remembering that the cranial cavity has an irregular shape and is bounded by curved surfaces, the value shown in the table can be considered satisfactory.

(d) *Correlation between cranial capacity and body-weight.*—Despite the fact that in the human subject the coefficient of correlation between brain and body-weight is extremely low (0.167 in male and 0.226 in female, Pearl, 06,) an intimate relation between these two characters found in the rat (Donaldson) suggests that in the rat at least it would be higher.

As a matter of fact the following coefficients² of correlation have been obtained:

TABLE VIII.

	Male.	Female.	Difference.
Body weight and cranial capacity...	.516 ± .074	.692 ± .058	.176 ± .094

Assuming that the regression between the body and brain weights can be expressed by a straight line with a given angle, the following equations were formulated and used to determine the correspondence between the predicted and observed values.

$$(1) \text{ Brain-weight, male} = (0.0072 \times (\text{body-weight, male}) + 9.349) \div 5.980.$$

$$(2) \text{ Brain-weight, female} = (0.0251 \times (\text{body-weight, female}) + 6.168) \div 6.009.$$

²Unfortunately in this series, several rats which were found dead are included and thus the correlation here obtained may be slightly less than it should normally be. It is however true that the normal fluctuation in body-weight is rather wide and therefore the including of several self-dead rats should not affect the results to any great extent.

5.980 and 6.009 are two factors for the transformation of the observed weight obtained from shot, into the estimated brain-weight. In other words, if we apply the characteristic equations which enable us to compare the cranial capacity in terms of the weight of shot from the observed body-weight, directly to the adult rats with known body- and brain-weight. (We have a large number of records which give the observed brain-weight accompanied by the corresponding body-weight) then the observed brain-weight was found to be $1/5.98$ of the weight of shot in the case of male and $1/6.009$ of the weight of shot in the case of female. This simple numerical relation between the observed brain-weight and shot-weight was found to be quite consistent and indeed the characteristic equations with these two new factors have given very satisfactory results in the test thus far.

(e) *Coefficients of correlation in man and rat.*—The coefficients of correlation between the cranial capacity and other cranial measurements in man have been determined by several investigators. The following table shows these relations in man as well as in rat:

TABLE IX.

CORRELATION BETWEEN CRANIAL CAPACITY AND LENGTH OF SKULL.

	Male.	Female.	Difference.	Investigator.
Aino893 ± .016	.663 ± .053	.230 ± .055	Pearson and Lee.
English597 ± .051	.691 ± .040	.094 ± .065	Macdonell.
German515 ± .050	.687 ± .037	— .172 ± .062	Pearson and Lee.
Naquada501 ± .054	.599 ± .039	— .098 ± .067	Fawcett.
Albino rat761 ± .039	.577 ± .062	.184 ± .073	Hatai.

CORRELATION BETWEEN CRANIAL CAPACITY AND BREADTH OF SKULL.

Aino561 ± .053	.502 ± .070	.059 ± .083	Pearson and Lee.
English631 ± .048	.646 ± .044	— .015 ± .065	Macdonell.
German672 ± .037	.707 ± .034	— .035 ± .050	Pearson and Lee.
Naquada434 ± .058	.532 ± .044	— .098 ± .072	Fawcett.
Albino rat838 ± .028	.632 ± .057	.206 ± .063	Hatai.

CORRELATION BETWEEN CRANIAL CAPACITY AND HEIGHT OF SKULL.

Aino544 ± .054	.521 ± .068	.023 ± .087	Pearson and Lee.
German243 ± .064	.451 ± .054	— .208 ± .084	Pearson and Lee.
Albino rat760 ± .039	.666 ± .053	.094 ± .066	Hatai.

CORRELATION BETWEEN CRANIAL CAPACITY AND (HEIGHT × BREADTH × LENGTH).

Aino795 ± .039	.780 ± .037	.015 ± .054	Pearson and Lee.
German701 ± .034	.814 ± .023	— .113 ± .041	Pearson and Lee.
Naquada674 ± .044	.793 ± .025	— .119 ± .051	Pearson and Lee.
Albino rat (height × breadth × length) ^{2/3}836 ± .028	.854 ± .026	.018 ± .038	Hatai.

As is shown in the table, the coefficients of correlation are higher in the rat than in the averages from the human subject in every case, except the cranial capacity and length of skull where the female rat is slightly low. The correlation is decidedly greater in the rat in the case of the capacity as related to the width and height, while in the case of the length and the product of the three diameters the results for the rat are close to those for man. Here again we notice that in the female the coefficients of correlation are slightly greater than in male in the case of the human skulls (except Aino) while in the rat the reverse is true. We can not lay too much stress on this relation, however, since as is shown in the column marked "difference" the size of the probable errors shows the differences to be without significance. Thus although, the general tendency is to show that in the human skull, except Aino, the coefficients of correlation are slightly greater in female nevertheless any definite statement must be postponed until we have data sufficiently abundant to further diminish the value of the probable errors.

(f) *Comparison between the observed and predicted values of skull measurements.*—In Table I we noticed that the mean values of the male characters are always greater than those of the female, the differences always being more than three times the probable errors. The greatest difference was found in the length of nasal bone, the least in the width of the cranium, while the remaining characters gave intermediate values. The question now arises: How the several characters will be related if the length of the entire skull of the male is reduced to that of the female? In other words, is the male skull to be considered as an overgrown female skull, or the female skull an undersized male skull? To answer this question a number of characteristic equations were prepared. These equations will enable us to determine the probable values of the characters in both sexes. The form of the characteristic equation is as follows:

$$Y = \bar{y} + r \frac{\sigma_y}{\sigma_x} (X - \bar{x})$$

where X , Y are the two characters under consideration, \bar{x} , \bar{y} are the two respective means of the arrays, σ_x , σ_y are also the two respective standard deviations and r is the coefficient of correlation. The following table was made in order to show the values of the characters when the lengths of the entire skulls were equated.

As is shown in Table X, when the length of the entire skull of the male is equated to the observed length of the female skull and vice versa, the sexual differences become very small. The closeness of agreement between observed and predicted values of the several characters varies with the

standard taken. This is due to the fact, as Tables IV.-VIII show, that the correlations is higher in male in some cases and in female in others. Five out of seven, characters in the male are absolutely greater than those in female even when the length of the entire skull in two sexes is equated. However the differences are too small to be significant, except in the case of the length of the nasal bone and perhaps the zygomatic width. The nasal bone is significantly longer in the male while the zygomatic width is slightly greater in the female. On the other hand if we equate the

TABLE X.

Probable values of male characters with mean skull length equal to that of the observed female.				Probable values of female characters with mean skull length equal to that of the observed male.			
Characters and No. of equations.	observed ♀	Probable ♂	Difference %	Difference %	Probable ♀	observed ♂	Characters and No. of equations.
Fronto-occipital length. I.	26.37	26.36	.07	-.89	27.51	27.26	Fronto-occipital length. II.
Squamosal distance. III.	15.06	15.09	-.19	.30	15.23	15.27	Squamosal distance. IV.
Height of skull. V.	11.14	11.21	-.67	1.69	11.30	11.49	Height of skull. VI.
Length of nasal bone. VII.	15.69	16.12	-2.64	1.81	16.65	16.96	Length of nasal bone. VIII.
Cranial capacity. IX.	10.37	10.42	-.50	.37	10.86	10.90	Cranial capacity. X.
Zygomatic width. XI.	20.93	20.68	1.17	-.80	21.92	21.75	Zygomatic width. XII.
(Height × length × width) ^{1/3} . XIII.	16.43	16.43	-.06	.53	16.79	16.88	(Height × length × width) ^{1/3} . XIV.

length of the female skull to that of the male then the greatest difference is noticed in the length of the nasal bone also and the height of the skull comes next. Besides these two the remaining characters give differences which are always less than 1 per cent. Taking all the results together, we reach to the conclusion that aside from the nasal bone, and perhaps the zygomatic width and height of skull too, the actual sexual differences in the remaining characters are inconsiderable, being less than 1 per cent. This suggests that the nasal bone in the rat may be considered as one of the secondary sexual characters. Consequently the female skull can not be considered as an undersized male skull nor the male skull an overgrown

female skull since these two skulls show at least one significant difference, i. e., in the length of the nasal bone (perhaps zygomatic width also). On the other hand the female cranium, i. e., if we disregard the length of the nasal bone and zygomatic width, may be considered as an undersized male cranium and vice versa, since the differences observed from the three measurements of the cranium in the two sexes are too small to be significant.

According to general belief the female brain is relatively heavier than that of the male although absolutely lighter. Blakeman, 05, found however, that "The Englishman of the same age, stature and diametral product as the mean woman has 1235 grs. brain-weight, or only 10 grs. more than the average woman. The Englishwoman of the same age, stature, and diametral product as the mean man has 1315 grs. brain-weight, or only 13 grs. less than the average man." He concludes from the above that "as far as present evidence goes, we can safely conclude that there is no sensible relative difference in the brain-weights of man and woman, the absolute differences observed are quite compatible with the differences which result from the relative size of the two sexes." The same conclusion, as has been given by Blakeman, may be drawn from the present study on the albino rats. It was found (see Table X) that when the length of the entire skull of the male rat is equated into the length of the entire skull of female, and vice versa, the resulting values for the cranial capacity in the two sexes are almost identical. The difference is in average less than 0.5 per cent, indicating that the sexual difference found in the cranial capacity is entirely accounted for the difference in the size of body. It is also interesting to note in our case that only one character has been equated and therefore if we took a multiple regression-equation the difference would probably almost disappear.

(g) *Characteristic equations.*—I have put together on the opposite page all characteristic equations which have been used in the course of the present study. Equations 1-14 will enable us to find the probable values of the other characters of the skull in two sexes when we know the length of the entire skull, while from the equations 15 and 16 we can obtain the probable brain-weight from the observed body-weight.

The characteristic equations show clearly that the relation between the length of the entire skull and the other characters of the skull, and brain and body-weight can not be determined by simple arithmetical proportion but require in each case the introduction of two or more of the necessary constants which are specific for the character chosen. It follows therefore that in general if the relation existing between the two characters turns

out to be skew (that is a non-linear regression) then the relation should be more complicated and a simple proportion would fail to correctly express the relations existing between the two characters under consideration. On the other hand if the regression is linear the relation may sometimes, but not always be shown by a simple proportion just as well as by a characteristic equation.

(1) Fronto-occipital length,	$\sigma = 0.5326$	Entire skull length,	$\sigma + 4.226$
(2) Fronto-occipital length,	$\varphi = 0.6663$	" " "	$\varphi - 1.311$
(3) Squamosal distance,	$\sigma = 0.1100$	" " "	$\sigma + 10.615$
(4) Squamosal distance,	$\varphi = 0.1004$	" " "	$\varphi + 10.884$
(5) Height of skull,	$\sigma = 0.1634$	" " "	$\sigma + 4.425$
(6) Height of skull,	$\varphi = 0.0937$	" " "	$\varphi + 7.246$
(7) Length of nasal bone,	$\sigma = 0.4911$	" " "	$\sigma - 4.285$
(8) Length of nasal bone,	$\varphi = 0.5619$	" " "	$\varphi - 7.654$
(9) Capacity of cranium,	$\sigma = 0.2790$	" " "	$\sigma - 1.172$
(10) Capacity of cranium,	$\varphi = 0.2863$	" " "	$\varphi - 1.527$
(11) Zygomatic width,	$\sigma = 0.6243$	" " "	$\sigma - 5.259$
(12) Zygomatic width,	$\varphi = 0.5830$	" " "	$\varphi - 3.298$
(13) (Height \times length \times width) ^{$\frac{1}{3}$} ,	$\sigma = 0.2590$	" " "	$\sigma + 5.672$
(14) (Height \times length \times width) ^{$\frac{1}{3}$} ,	$\varphi = 0.2119$	" " "	$\varphi + 7.619$
(15) Brain-weight,	$\sigma = (0.0072$	Body-weight,	$\sigma + 9.349) \div 5.980$
(16) Brain-weight,	$\varphi = (0.0251$	" "	$\varphi + 6.168) \div 6.009$

CONCLUSIONS.

1. All the characters here examined are absolutely greater in the adult male than in the adult female.

2. The coefficients of variation reveal the fact that the male characters show a general trend to a greater degree of variability than those of the female. The length of the nasal bone and the zygomatic width show a much greater variation than any other skull characters in the two sexes.

3. The coefficients of correlation are always positive and tend to be higher in male than in female. The correlation between cranial capacity and body-weight was found to be quite high (0.516 in male and 0.692 in female).

4. The brain-weight corresponding to the observed body-weight may be calculated from the following two characteristic equations:

Brain-weight, male = $(0.0072 \times (\text{body-weight, male}) + 9.349) \div 5.980$.

Brain-weight, female = $(0.0251 \times (\text{body-weight, female}) + 6.168) \div 6.009$.

5. The observed sexual differences are considerably reduced when the length of the entire skull is equated to either the male or female standard. When the lengths of the entire skull in the two sexes are equated and

the remaining characters are compared, the greatest difference is found in the length of the nasal bone (mean differences amount to more than 2 per cent), the height of skull and width of zygoma come next (slightly over 1 per cent), while the smallest differences are found in the remaining characters (less than 1 per cent). From the relation shown above the writer inclines to consider the relative development of the nasal bone in the rat as one of the secondary sexual characters.

6. From the above it is clear that the female skull can not be considered as an undersized male skull, nor the male skull as an overgrown female skull, since there is at least one significant difference in the skulls of the two sexes; i. e., the length of the nasal bone.

7. The female cranium on the other hand may be considered as an undersized male cranium, and vice versa, since the differences found from the three cranial measurements in the two sexes are too small to be significant.

8. The relation between the coefficients of correlation and regression is linear.

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REPTILIAN EPIPHYSES.

BY

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WITH 24 TEXT FIGURES.

It has long been the opinion of anatomists that bony epiphyses are confined exclusively to the skeleton of the Mammalia. Among other features often given as characteristic of the mammals is the one that epiphyses occur on the bones.

Dollo (1) in 1884 first called attention to the fact that real epiphyses are found in the reptiles, although Albrecht (2) had the year before published a short note on epiphysial structures of the vertebral spines of *Sphenodon*. Dollo gave a complete summary of the literature on the subject and a list of epiphyses which he observed in seven families of the lizards and in *Sphenodon*. He promised a more complete description with illustrations but I have not been able to ascertain that his promise has been fulfilled. Parsons (3) writing twenty-two years later, has revived the interest in epiphyses of the reptiles in connection with his studies on the epiphyses of the human skeleton.

At the suggestion of Dr. Williston the study of epiphyses as they occur in the reptiles was taken up in an attempt to solve the problem of the relationship of the Chelonia and Plesiosauria as evidenced in the so-called epiphyses of the two groups. The methods pursued are those referred to in a previous contribution on the lizard sacrum (4) and which are given below in full. These methods of clearing animal tissues were first introduced into America by Dr. Mall and were first fully outlined by him in his study of ossification centers in the human embryo (17). Hill (18) further outlined the methods and gave their application to subjects other than the study of osteology. The method consists principally in the use of KOH as a clearing agent on objects too large for the ordinary clearing agents to act upon. This was the method pursued by Schultze (20) who was its originator, and it is usually known as the Schultze method.

Embryos which have been freshly collected should be placed in 95 per cent alcohol, which acts both as a killing and fixing agent. If the embryos are of large size the alcohol should be changed frequently until the tissues of the specimen are thoroughly shrivelled and hard. For pig embryos of about one-half term, ten days is necessary for proper fixing. Smaller and larger objects can be fixed for a length of time based on the time mentioned. Adult animals are also to be fixed in strong alcohol, 95 per cent serving as well as does absolute alcohol as Hill states. It has been possible to clear quite large animals, both adult and embryonic. I have a cow embryo over five inches in length, the tissues of which are perfectly transparent. I also cleared a small garter snake of nearly two feet in length, and an adult *Heloderma*, so that the method has a wider application than was at first supposed. For objects which have been fixed in any of the acid fixing agents it is best to place them in strong alcohol in which is a strong solution of iodine. This removes the acid and allows the KOH to take effect better. Before using the iodine on such objects it had been found almost impossible to clear satisfactorily, and if cleared at all there still remained a hazy appearance which nothing served to remove. I have just recently received some alligator embryos from Professor Reese, which had been fixed in acetic acid and after several days in the iodine alcohol, they are now beginning to clear nicely. Stains in the tissues are best removed with strong ammonia as Mall and Hill state. Stronger oxidizing agents have been tried but the bubbles formed in the tissues by the more powerful agents are nearly impossible to remove.

Specimens preserved in formalin, I find, clear with uncertain rapidity. Usually it takes a formalin hardened specimen in 10 per cent KOH from three to six weeks to clear but recently I have had some formalin hardened turtle embryos to clear beautifully over night. The embryos had been fixed for at least two months in formalin and were in strong alcohol for about a week before clearing. Just what is the cause of this sudden clearing in objects which have been considered difficult is not apparent.

Objects cleared in KOH should be placed in glycerin diluted with water. Mall suggests the addition of an alkali to kill fungi, but I do not find it necessary. After removing the stains with ammonia the objects are then to be placed in pure glycerin to preserve them permanently. If kept in glass or rubber-stoppered bottles they will keep indefinitely. Cork turns the glycerin brown.

Objects cleared in this way are readily photographed. Sunlight does not serve well for an illuminant on account of the shadows cast. It has been found, after repeated trials, that a strong arc light thrown on the

specimen at an angle of about forty-five degrees with a reflector on the opposite side so as to illuminate the object uniformly gives the best results. The specimens are placed in a broad, shallow glass dish with just enough clear glycerin to cover them. The dish should be raised somewhat on small blocks so as to throw the background out of focus. Two of the half-tones in the present essay were prepared in this way.

For the purpose of determining the presence or absence of epiphyses in the Reptilia the following groups have been investigated and will be discussed in the order named: Chelonia, Plesiosauria, Crocodilia, Lacerilia and *Sphenodon*.

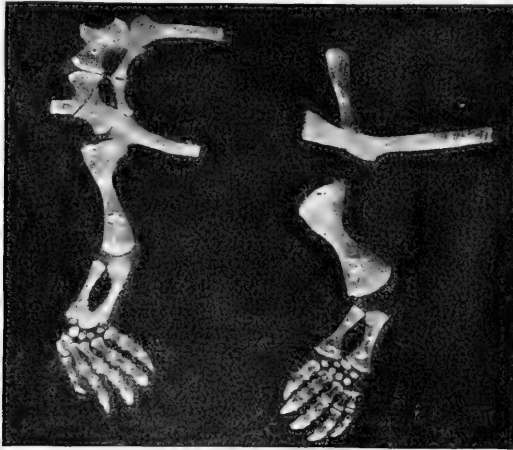


FIG. 1. Limbs and girdles of a young *Chelydra* 44 mm. in length; no epiphyses. $1\frac{1}{4}$ natural size.

The turtles studied consist of embryos of *Chelydra*, *Graptemys*, *Chrysemys*, *Trionyx* and *Aromochelys*, the young of *Chelydra serpentina* Linné and *Chrysemys picta* Hermann, together with adult skeletons of many genera, notably *Chelydra*, *Chrysemys*, *Testudo*, *Chelys*, *Chelone*, *Cistudo* and *Trionyx*.

Very little is to be learned in regard to epiphyses from the embryos of the turtles. In the lizards where epiphyses are found they are not formed until after the time of hatching from the egg. They are seen as minute points in the cartilage at the ends of the limb bones of a young lizard, *Sceloporus*, two days old. The turtle specimens which were investigated particularly for the presence of epiphyses, range from very small embryos in which the carapace is not yet formed to and including the stages of

hatching and subsequent stages. In none of the specimens, although I have examined them with the greatest care, have I been able to find the slightest indication of bony epiphyses on any of the skeletal elements. The embryo turtles, when cleared, show the ossificatory centers of the bones very beautifully, but there is never any separate center for an epiphysis. If epiphyses are to be found in the turtles they should be present in the young animal. In a specimen of *Chelydra serpentina* Linné, 44 mm. in length, the limb bones (Fig. 1) show no differences from the limb bones of an adult *Chelydra* excepting, of course, in size. We should certainly expect epiphyses to be evident at this stage, if ever. They are not present in a slightly younger specimen of *Chelydra*, nor are there any evidences of them in a more advanced *Chrysemys*.

Smith Woodward (5) states: "Other characters, such as the conical epiphyses of certain limb bones, also seem to imply community of origin of the Chelonia with the Sauropterygia and Batrachia"; and Parsons (6) states in parenthesis: "I think that I have found traces of an epiphysial line in the great trochanter of the Chelonia, but am not sure of it." The "conical epiphyses of certain limb bones" were first noted by Seeley (21) in his essay on *Pariasuarus* where he says: "All the long bones of the Nothosauria and Plesiosauria ossify in the same way as the long bones of living frogs, and consist of cylindrical girdles into which long, conical epiphyses penetrate, so as to meet, or nearly meet, in the middle of the shaft, from which they are often easily or naturally separated. I have had no opportunity of determining whether this condition is present in the long bones of Labyrinthodonts, and I only otherwise know it as a rare condition in some of the long bones of Chelonia from the Cambridge Greensand, and in an undescribed epiphysis, which I believe to be Dinosaurian, from the Oxford Clay, and the proximal end of the tibia of *Protorosaurus*." In his essay on *Protorosaurus* Seeley (22) practically repeats the same facts.

From the above quotation it is evident that Seeley had a correct idea of the ossification of the reptilian bones. The term epiphyses was used, it seems to me, inadvertently to express the nature of the elements and not to express the idea of any homology of the reptilian structure with the familiar elements in the mammals. Later authors following Seeley quoted his words but missed the idea and hence has arisen the present discussion. Osborn (23) mentions epiphyses as possible evidences of relationship between the turtles and plesiosaurs. The fact remains, however, that *there is not the slightest evidence either embryologically or anatomically of any bony epiphyses on any of the skeletal elements of the Chelonia that*

I have examined. To be sure there are in the young of the turtles pads of cartilage which form the articular surfaces such as are found on the limb bones of the Amphibia, but in the turtles there is no distinct separation of the cartilage from the diaphysis as in some of the Amphibia, nor is there any trace of any bony structure. The shaft is entirely ossified from its center of ossification and the cartilaginous pad becomes in time fully osseous by such a process. The pad of articular cartilage often makes a distinct line on the bone which becomes evident when the cartilage is macerated. I have noticed such lines on the limb bones of the alli-

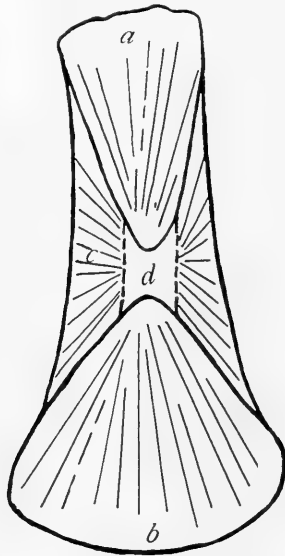


FIG. 2. Longitudinal section of a propodial of a Sauropterygian; from the Kimmeridge Clay of Ely. *a* = Endochondral bone (Proximal epiphysis). *b* = Endochondral bone (Distal epiphysis). *c* = Perichondral bone (Shaft). *d* = Medullary cavity. $\frac{1}{4}$ natural size. After Lydekker. (Lydekker's interpretations in parentheses.)

gator and various turtles. Doubtless the line to which Parsons refers is due to the articular cartilage on the great trochanter.

In the Plesiosauria there have long been known some propodial bones of young animals which offer very puzzling characters. One of these specimens (Fig. 2) is in the British Museum and was figured diagrammatically by Lydekker in his Catalogue of fossil Reptiles and Amphibia (7). Parsons has also given a diagram of a young propodial, whether of the same bone or not I do not know. Another well known specimen is the

one described and figured by Williston (8). I have studied this propodial and it offers some very peculiar characters. As described by Williston there are two cones of osseous matter whose apices do not quite meet at the center but are separated by a small medullary cavity into which four canals from the outside enter.

The cones of this embryonic propodial (Fig. 3) are represented in the diagram (Fig. 4). Williston says: "The . . . specimen discloses these epiphyses (cones) with a smooth rounded surface . . . the outer

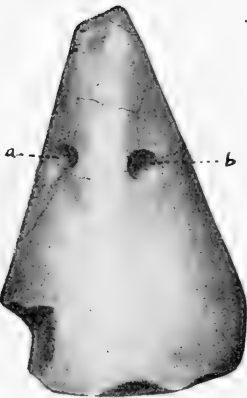


FIG. 3.

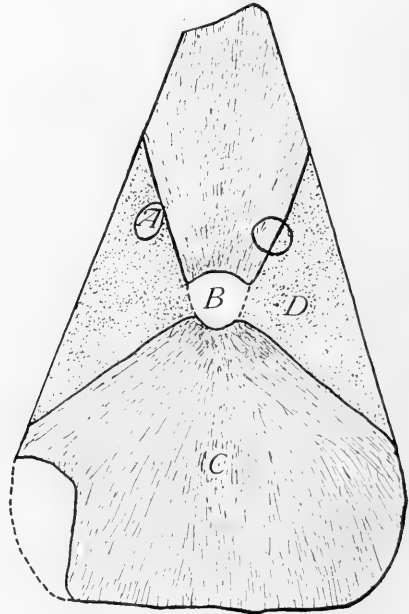


FIG. 4.

FIG. 3. Embryonic plesiosaur propodial. Foramina at *a* and *b*. $\frac{1}{2}$ natural size. After Williston.

FIG. 4. A section through the embryonic propodial shown in Figure 3. *A* = Foramen of the nutrient canal. *B* = Medullary cavity. *C* = Cone of the endochondral bone. *D* = Perichondral bone. $\frac{1}{4}$ natural size.

part peeling away as does the bark from a tree." The structure of this propodial is especially dense and the same has been described for many other bones of young plesiosaurs. The denseness of the tissue is one of the peculiar characters of these cones and persists throughout the life of the individual. I have made sections of several propodials to determine the fate of the cones and find them clearly marked in the propodial of a half-grown individual (Figs. 6 and 8). In this bone there also persists

a remnant of the medullary cavity and a single small canal. On the external surface of the adult propodial (Fig. 7) there is no evidence of the cones or of the canals, but on sectioning an adult humerus (Fig. 9) the cones are seen to be represented by dense areas of tissue at the ends of the bone. The areas are without definite boundaries and merge gradually into the texture of the rest of the bone.

The cavity which lies between the apices of the two cones is the medullary cavity. Its presence in the embryonic bone can be accounted for only

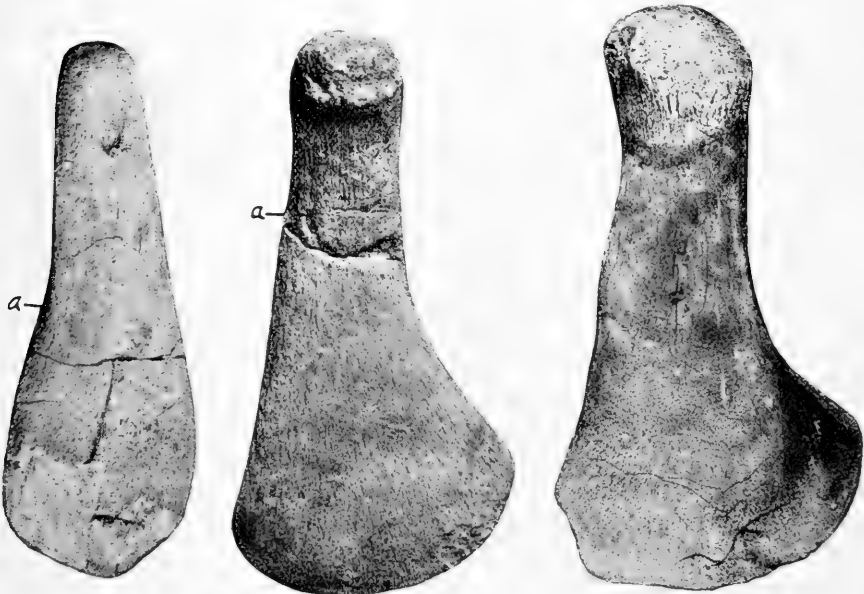


FIG. 5.

FIG. 6.

FIG. 7.

FIG. 5. An immature plesiosaurian propodial showing foramen at *a*. $\frac{7}{10}$ natural size. After Williston.

FIG. 6. Propodial of half-grown plesiosaur showing the foramen at *a*. $\frac{1}{3}$ natural size.

FIG. 7. An adult plesiosaurian humerus seen from the dorsal side. There are no foramina or cones evident. About $\frac{1}{4}$ natural size.

on the presumption that the ancestors of the plesiosaurs had hollow limb bones. The cavity is certainly a vestigial one. It is quite large in the embryonic bone (Fig. 4). There is a small cavity filled with calcite in the propodial of the half-grown individual (Fig. 8). In the adult bone (Fig. 9) the cavity has entirely disappeared. From these facts it is apparent that the cavity is a transitory structure and is on the same plane

as the persistence of the gill clefts in the mammalian embryos. The ancestors of the plesiosaurs are yet to be discovered. When they are brought to light they will probably be found to have hollow limb bones.

The canals and the foramina of the propodials are not so readily accounted for. The presence of canals in the middle of a limb bone is a very remarkable character and is without parallel among the known vertebrates. The canals are probably for the passage of blood vessels, but why

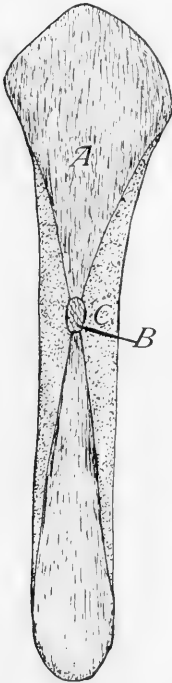


FIG. 8.



FIG. 9.

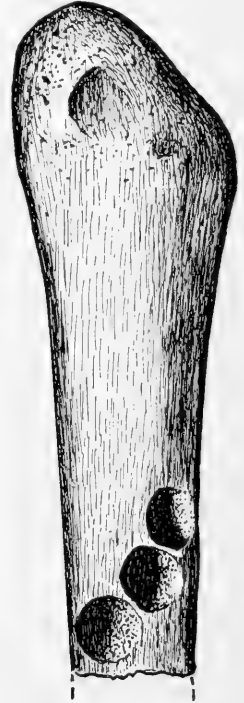


FIG. 10.

FIG. 8. A section through the plesiosaurian propodial shown in Figure 6. *A* = Endochondral bone. *B* = Medullary cavity filled with calcite. *C* = Peri-chondral bone. $\frac{3}{5}$ natural size.

FIG. 9. A section through the humerus of an adult plesiosaur. $\frac{1}{4}$ natural size.

FIG. 10. Proximal portion of a young plesiosaur propodial showing three foramina. Seen from the edge. $1 \frac{1}{10}$ natural size.

they should be so large and why they should disappear in the adult, is inexplicable. The nutrient foramina, if such they are, have been observed in many specimens. Williston describes four in the embryonic propodial, there are three present in a more mature bone before me (Fig. 10), one

in another, and there is but a single foramen present in the element figured by Williston on Plate XXIII of his *North American Plesiosaurs* (Fig. 5). Kiprijanoff has figured an immature propodial (Figs. 12 and 13) from Russia, in which there is a single foramen present. It is thus evident that the canals and their foramina are very variable in number and there is also a variation in their position on the bone. In the embryonic propodial they open out on both the dorsal and ventral surfaces (Fig. 3). In the propodial shown in the photograph there is a single foramen evident on the dorsal surface (Fig. 6). In all other specimens with which I am acquainted the foramina open out on the edge of the bone (Figs. 5 and 10). From these facts it would appear that the blood

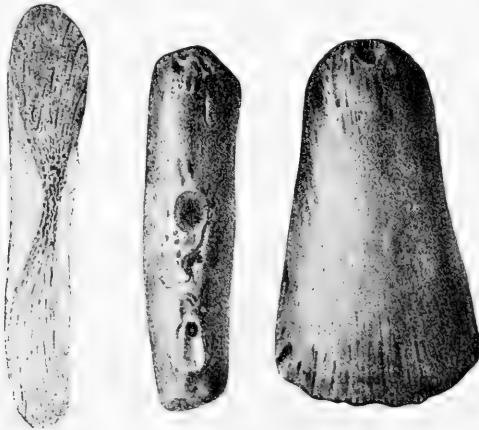


FIG. 11.

FIG. 12.

FIG. 13.

FIG. 11. A longitudinal section through an immature propodial of a plesiosaur showing the cones and the perichondral sheath. $\frac{3}{4}$ natural size. After Kiprijanoff.

FIG. 12. An immature propodial showing foramina. Seen from the edge. $\frac{3}{4}$ natural size. After Kiprijanoff.

FIG. 13. Same bone seen from the dorsal aspect. $\frac{3}{4}$ natural size. After Kiprijanoff.

vessels which the canals contained were veins and not arteries. That arteries are among the more constant structures in the body of the vertebrates is well known among anatomists, and where inconsistencies occur in blood vessels it is usually among the veins. I have made sections of several immature propodials in the hopes of finding some clue to the meaning of the foramina and canals, but nothing can be stated here other than that they are present in the young and absolutely disappear in the adult. The bone fibers around the foramina in one section are seen to have a spiral arrangement (Fig. 14).

Our main interest in these propodials is the fact that the cones of osseous matter (Fig. 11) described above have frequently been called epiphyses. But when these plesiosaurian structures are compared with the true epiphyses of other animals, they are found to be widely different. Epiphyses are always at the ends of the long bones and never go to form any part of the shaft. These cones in the plesiosaurs form part of the diaphysis of the propodial, and are to be compared to the similar

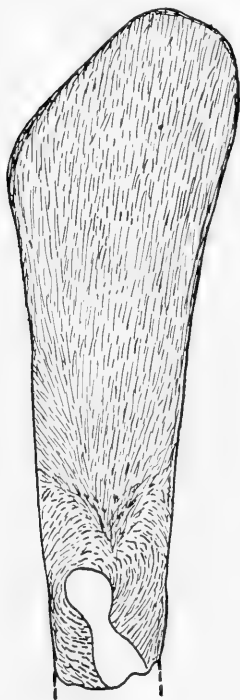


FIG. 14.

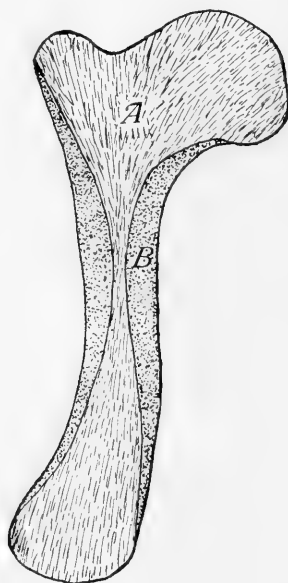


FIG. 15.

FIG. 14. A section through the bone figured in "10" showing the spiral arrangement of the bone fibers. $1 \frac{1}{10}$ natural size.

FIG. 15. Section through the femur of an adult turtle, *Trionyx*. The endochondral bone A is clearly distinct from the perichondral bone B. $1 \frac{1}{2}$ natural size.

structures found in the limb bones of the turtles, lizards and crocodiles (Figs. 15, 16 and 17). The peripheral portion of the plesiosaur bone which is called the shaft by Lydekker, is the periosteal ossification, and goes to form a portion only of the diaphysis. The cones which Lydekker calls epiphyses are the endochondral bone evident in all embryos of the

Sauropsida. The cones are not confined to the long bones, but occur in the ribs and all of the elements of the appendicular skeleton (Fig. 18). Parsons in his last article on epiphyses (10) draws a comparison between the conical ossification at the ends of the plesiosaur bones and the conical cartilaginous ends of the developing long bones of the pigeon. This comparison is a new and interesting one and contains the key to an exact interpretation of these structures in the plesiosaurs. Such an appearance

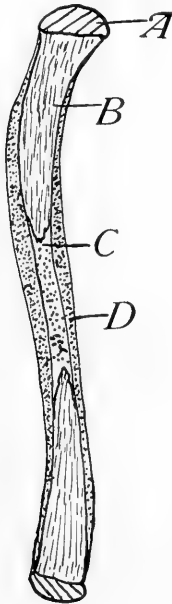


FIG. 16.

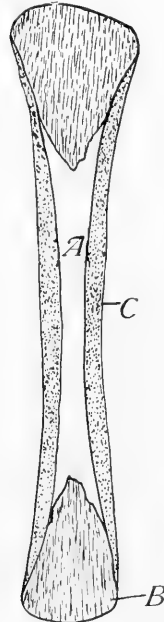


FIG. 17.

FIG. 16. A section through the humerus of *Amblyrhynchus*. A = Bony epiphysis. B = Endochondral bone which is less compact than the perichondrium. C = Medullary cavity partly filled with bone fibers. D = Compact perichondrium. $1\frac{1}{2}$ natural size.

FIG. 17. A section through the tibia of *Alligator*. A = Large medullary cavity. B = Cone of endochondral bone. C = Perichondrium. $\frac{2}{3}$ natural size.

is not confined to the pigeon for I have noticed the cones of cartilage in the developing bones of the lizards, the turtles, and the chick. The cones are due to the mode of ossification in the Sauropsida, which is widely different from the method of ossification in the Mammalia. The formation of the endochondral bone lags far behind the perichondral portions in the Sauropsida. The perichondrium is formed as a sheath around the

entire endochondral part. Brachet gives an account of the development of this cone of cartilage in the chick (11) and I think his account will apply to other Sauropsida. The cone of cartilage is first calcified and the calcified cartilage is subsequently replaced by bone. The formation of the endochondral bone takes place after the formation of the perichondral portion. The perichondral bone is what Lydekker in the plesiosaurs has called the shaft, and the endochondral portion or the cones he has called the epiphyses. Both of these interpretations are incorrect. The cones or endochondral, and the sheath or perichondral portion together, form the diaphysis, and there is no epiphysis on the long bones of the plesiosaurs. The fact that the cones are separate from the perichondrium in the fossilized remains of the plesiosaurs is due, no doubt, to the fact that the



FIG. 18. Photograph of the foot of a chick of sixteen days incubation. The cones of cartilage are clearly evident in the metatarsals and the phalanges. $1\frac{1}{4}$ natural size.

endochondrium forms subsequent to the formation of the perichondrium. The perichondral sheath is almost fully formed before the endochondral cone starts to ossify.

Brachet has fallen into the same misconception of the conical growth at the ends of the bones in the chick as did Lydekker in the case of the plesiosaurs. He calls the endochondral portion an "epiphysis," but it is not epiphysial in any structural sense for epiphysis means a *growth upon* and the structure in this case is certainly not upon, but within, the upper portion of the perichondrium.

The cones or endochondrium in the plesiosaurs have been called epiphyses and have been used as a character on which to base the relationship of the turtles and plesiosaurs. The question of this relationship is an old one and has been discussed by various authors. But the presence of

epiphyses in the two groups cannot be used as a criterion of relationship since neither has these structures. It might be more logically claimed that they are related because of the absence of epiphyses. But the presence or absence of these elements, it seems to me, is of little value. If it is of value, then the lizards and mammals are related, for epiphyses are abundantly present in both groups. That epiphyses are not good diagnostic characters for the distinction of large groups of vertebrates is well shown by the fact that they occur in the lizards and that here they may be abundantly present or almost entirely absent. They may be a good generic or family character, but this point requires further investigation. Some lizards (*Chameleon*, *Heloderma*, *Amblyrhynchus*) have a great number of epiphyses, some (*Phrynosoma*, *Eremias*) have but few. *Draco*, apparently, has none.

I find no epiphyses in the crocodiles. I have examined young specimens of the *Alligator mississippiensis* Daudin, five inches in length from the tip of the snout to the base of the tail, and have seen no indications of any epiphysial growth. I have also examined adult skeletons of the garial, the Florida crocodile, and the Mississippi alligator, but have seen nothing indicative of epiphysis, although there is present a line due to the cap of articular cartilage on the ends of the limb bones.

The lizards offer an interesting field for more extended investigation on this subject. Epiphyses occur abundantly in some forms and principally at both ends of the limb bones, on some of the girdle bones, on some of the carpals and tarsals, on both ends of the metacarpals and metatarsals, at the proximal end of the phalanges, on the spines of the vertebrae, and Dollo has reported them on the skull and ribs in certain forms, although I have not been able to find them on any of the skulls at my disposal, except that epiphysial structures are present on the posterior end of the mandibles of *Amblyrhynchus*.

The epiphyses of the lizards are not always bony. Some are merely calcified cartilage while a few are true bone, as Parsons has shown in *Iguana*. I have made sections of the epiphysial regions of *Amblyrhynchus* and have definitely ascertained that there are Haversian canals and lacunae present in the epiphyses. The fact that some of the epiphyses are not bone is, however, a matter of but slight importance. The important fact is that there are distinct structures present in the lizards which correspond with the similarly placed epiphysial structures in the mammals.

The lizards examined are the same as I have listed in a previous contribution on the lizard sacrum and the reader is referred to that

article for the forms discussed here. In addition to the forms there mentioned I have recently examined the skeletons of two specimens of *Amblyrhynchus cristatus* Gray, of the family Iguanidae, from the Gallapagos Islands; one specimen of *Eremias pulchella* Gray, of the family Lacertidae, from Africa; and a skeleton of a young *Varanus*, of the family Varanidae.

In the embryos of *Cnemidophorus sexlineatus* Linné there are no epiphysial ossifications. In three young specimens of *Sceloporus chrysostrictus* Cope, 24 mm. in length from the tip of the snout to the base of the tail, which are two days old, the epiphyses can be detected as minute points in the cartilaginous pads at the upper and lower ends of the humerus and femur, and at the distal ends of the metacarpals and metatarsals. In a slightly older specimen of *Sceloporus* there have appeared at the distal end of the humerus four epiphysial centers. It is not at all unusual for more than one center to appear at the end of a long bone. On the humerus of *Phrynosoma* there are two centers on the distal end, and on the upper and lower ends of the humerus of *Amblyrhynchus* there are four and five centers respectively. In an adult specimen of *Sceloporus* the epiphyses have become almost indistinguishably united with the diaphyses. In this specimen, also, there appears the ununited olecranon, which was not apparent in the young. The olecranon in the lizards is of separate origin from the diaphysis and only unites with it late in life. In his paper on "Traction Epiphyses" Parsons has figured the developing olecranon of *Iguana* and discusses its probable origin and its homologue in the higher vertebrates.

In the young specimen of *Phrynosoma*, 26 mm. in length, there can be detected at the distal end of the humerus two epiphysial centers and one on the proximal end. One epiphysis is present on the distal end of all of the metacarpals. There are no epiphyses either on the radius, ulna, carpals or phalanges. One epiphysis is present on the upper and one on the lower end of the femur. As in the fore limb, there are no epiphyses present on any of the other bones of the leg, but epiphyses are present on the distal end of the metatarsals. In the adult specimen of *Phrynosoma*, 46 mm. in length, the epiphyses are almost indistinguishably united with the diaphyses. The olecranon has not, however, joined the ulna.

In the young specimen of *Iguana*, 73 mm. in length, there is one epiphysial center at each end of the humerus, one on each end of the ulna, none on the radius (Fig. 24). One epiphysis is present on each end of the femur, tibia and fibula, and one at the distal end of all of the metacarpals and metatarsals. There are no apparent epiphysial structures

on the phalanges excepting some very minute points of bone which appear in the articular cartilage of one or two.

So far as can be detected from my single specimen, *Draco volans* Linné, 62 mm. in length, has no epiphyses, but it is possible that they would be evident in the young. The olecranon is free.

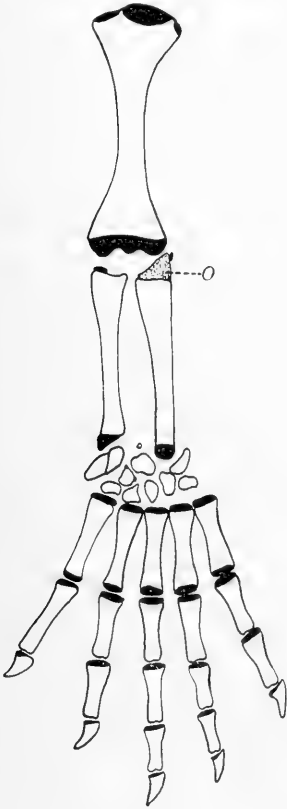


FIG. 19.

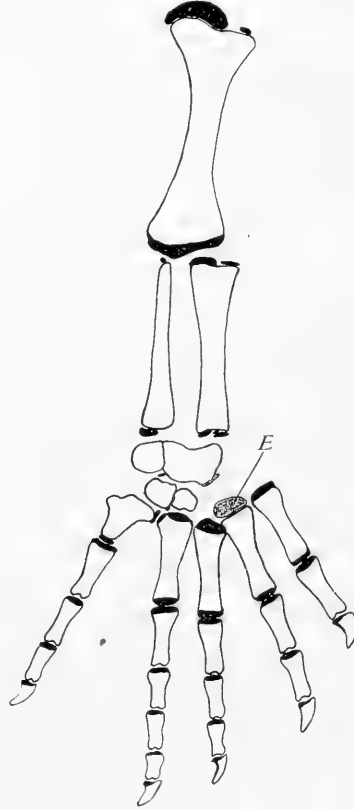


FIG. 20.

FIG. 19. The fore limb of *Heloderma suspectum* Cope. The shaded parts represent the epiphyses. *O* = the olecranon. There are two epiphyses on the os centrale. Twice natural size.

FIG. 20. The right hind limb of *Heloderma suspectum* Cope, seen from above. The epiphysis *E* is probably an element of the tarsus which has become united with the metatarsal. The tibiae has two epiphyses which probably represent atrophied elements of the tarsus. Twice natural size.

In the young specimen of *Chamelecon owenii* Gray, 44 mm. in length, there are distinct bony epiphyses on each end of the humerus, radius and

ulna. At this stage there are none apparent on the metacarpals or phalanges. There is a distinct center at each end of the femur, tibia and fibula, but none on the metatarsals or phalanges. In the adult of the same species, 135 mm. in length, the epiphyses are fully as distinct as they are in the young, with the exception of that at the head of the humerus, which appears to be partly fused with the shaft. In the adult form there are epiphyses in the hand and foot.

In the adult specimen of *Heloderma suspectum* Cope, the epiphyses are abundant and in places quite mammalian in appearance. The upper end of the humerus has a small oval epiphysis in the center with a smaller one on each tuberosity. That at the distal extremity of the humerus is quite broad and has a particularly mammalian aspect, being quite long from side to side, entirely covering the lower end of the bone. It is divided into three condyles by two trochlear grooves. The epiphysis at the upper end of the ulna is quite like a mammalian olecranon. The one



FIG. 21. The left mandible of *Amblyrhynchus*. The shaded part *E* represents the epiphysis. *A* = Angular. $1\frac{1}{3}$ natural size.

at the lower end is disc shaped. The radius has small, poorly developed, calcified epiphyses at each end. In the hand the metacarpals have epiphyses at each end and they are present at the base of all of the phalanges (Fig. 19). On the upper and lower end of the femur there are epiphyses, the lower one having a mammalian aspect. The tibia has a small epiphysis on each end and the same condition holds for the fibula. On the tibiae there are two small ones and one also on the fibulare. In the foot, as in the hand, there are epiphysial structures on each end of the metatarsals, with the exception of the first, and one at the base of all of the phalanges (Fig. 20). I have not been able to detect epiphyses on the girdle bones of this form, although Dollo has reported them on some of the forms examined by him. Siebenrock has seen epiphyses on the ilia of *Uroplates*.

There are in many of the epiphyses of *Heloderma* certain resemblances to similarly placed structures of the mammals. This is very remarkable as being the only case in which there is such a resemblance among the

many forms I have examined. I know of no way to account for it save by parallel development. What the causes are which have produced this peculiar development in these lizards is obscure. I know of nothing in their habits of life which could bring about such a development, since they are but slightly different in their mode of life from many other lizards which have the regular reptilian structure.

The epiphyses are more abundant on the skeleton of *Amblyrhynchus cristatus* Gray than on any other form I have examined. There is a small



FIG. 22. Humerus, ulna and radius of *Amblyrhynchus*. $1\frac{1}{2}$ natural size.

one on the proximal end of each mandible, on the angular (Fig. 21), but none can be detected elsewhere on the skull. On the humerus of this form there are four epiphysial centers on the upper end and five on the lower (Fig. 22). The four on the upper end are of unequal size and only one of them is ossified. The other three are calcified cartilage. The ossified epiphysis occupies the head of the humerus, two small ones of calcified cartilage occur on the large tuberosity, and one on the lesser tuberosity. The five at the lower end are also of unequal size and only one of them is ossified. The large central bony one resembles the trochlear epiphysis of a mammal, but the trochlea is itself small. There are three

of calcified cartilage on the external condyle and one on the internal condyle. The upper and lower ends of the radius and ulna have epiphyses. The upper end of the ulna has also an olecranal ossification which, in an older specimen, has become united with the epiphysis. There are no epiphyses on the carpals. On the metacarpals, however, they are found at each end. At the base of the proximal phalanges they are prominent structures, but are not distinguishable at the base of the distal phalanges. The head of the femur has an epiphysis and there is one on the trochanter



FIG. 23. Femur, tibia and fibula of *Amblyrhynchus*. $1\frac{1}{2}$ natural size.

(Fig. 23). On the lower end of the femur there are three epiphyses, none of which appear to be ossified. Each end of the tibia and fibula is provided with a bony epiphysis. There is a small one on the tibiale, two small ones on the fibulare. The condition in the foot is the same as in the hand. There are distinct, bony epiphysial structures on the spines of the posterior cervical and anterior dorsal vertebræ of this form. No epiphyses have been found on the centra of any reptile so far as I am aware. It is of interest to note in this connection that Beddard quotes Parker as an authority for the statement that there are epiphyses on the centra of some of the parrots.

In the skeleton of *Eremias pulchella* Gray which I have recently examined the only epiphyses which can be detected are on the upper end of the humerus and at the distal end of the metacarpals and metatarsals.

The skeleton of *Varanus* shows such close resemblances in the arrangement and position of the epiphyses to the skeletons of *Amblyrhynchus* and *Heloderma* that one is forced to the conclusion that they are closely related members of a natural group of the lizards. This relationship

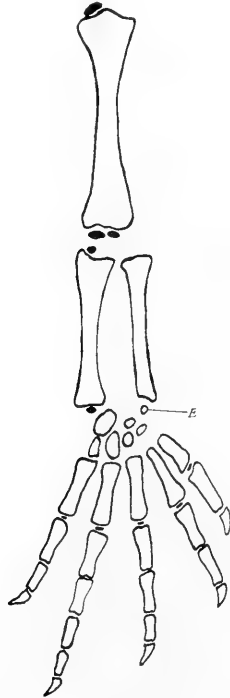


FIG. 24. The right fore limb of a young *Iguana* showing the manner in which epiphyses arise. They are not connected with the diaphyses in the early stages of growth. *E* = Epiphysis? Carpal? 2 5/7 natural size.

has been claimed on other grounds and the arrangement of the epiphyses tends to corroborate this view. As in the two forms mentioned, there are in *Varanus* epiphyses present in abundance on the skeletal elements. Each end of the long bones of the limbs, each end of the metacarpals and metatarsals, the bases of all of the phalanges, including the ungual phalanges as in *Heloderma*, and some of the tarsal and carpal bones, are provided with epiphyses.

It is interesting to note the method of development of the epiphyses in the lizards. As above stated there are no epiphyses present in the embryos of the lizards so far as I am able to determine. In the embryos of *Cnemidophorus* there is not a trace of epiphysial structures in the articular cartilages of the limb bones. In a young *Sceloporus*, the epiphyses are just appearing as minute points. In the adult *Sceloporus* the epiphyses are indistinguishably united with the diaphyses. This seems to be the usual life history of the epiphyses among the Lacertilia. But to this there are some notable exceptions. In the young of *Chameleon owenii* Gray, there are in a young specimen, two inches in length, no epiphyses present in the hand or foot. The epiphyses in the hand and foot of the adult are quite large and do not unite at all with the diaphyses, but remain much more distinct than I have ever seen them in any mammal. I do not possess young stages of the *Heloderma*, but in the young of *Amblyrhynchus* and *Varanus* the epiphyses separate easily from the diaphysis and this is especially true of the *Varanus*. In the adult forms of this genus the epiphyses are closely attached but do not fuse with the shaft.

From the above facts it will be clear that the method of development of the epiphyses in the Lacertilia is not at all uniform. In some lizards the epiphyses are more evident in the young; in others they are more evident in the adult. Whether these facts are of any particular significance or not remains to be determined. At any rate it is clear that the presence of epiphyses in the Lacertilia is a very variable matter and one would be inclined to the opinion that they may occur at any place on the skeleton where there is strain or pressure. Epiphyses are to a certain extent sesamoid, but are not sesamoid bones in the ordinary meaning of that term.

One of the most interesting facts in regard to the epiphyses of the Lacertilia is that they are never found on both ends of the phalanges. In none of my specimens is there the slightest indication of an epiphysis on the distal end. They occur always on the proximal end. In the mammals, with the exception of the Cetacea, the same condition is found. They are present only on the proximal end of the phalanges in both groups. Perhaps this is another case of parallel evolution, but it doubtless has more significance than that. It seems to me that the epiphyses and their arrangement is a matter of some importance in the phylogeny of the vertebrates. Not that they can be of much taxonomic importance, but that they are significant of primitive conditions. It has always been assumed by anatomists that epiphyses were developed first in the mam-

mals, but we now know that they are present in very generalized reptiles. When we come to know the primitive Reptilia better the meaning of the epiphysial structures will be more clearly understood. It is evident that epiphyses are of various origins. Some arise undoubtedly through use, some represent atrophied elements, and some appear to arise from other causes. I am of the opinion that the epiphyses of the Lacertilia are inherited structures which they have obtained from their Rhynchocephalian ancestors and the fact that the arrangement of these structures in the lizards and mammals is the same cannot, it seems to me, be due to like causes. There is some real fundamental cause for such an arrangement. Why epiphyses develop in some forms and not in others is another question and can have no bearing on this suggestion.

Broom (12) has recently called attention to the arrangement of the epiphyses in the human hand and foot where they occur at the base of all of the phalanges, and at the distal end of the metacarpals and metatarsals of digits II, III, IV and V, but at the proximal end of the first metacarpal and metatarsal. He suggests that possibly the first metacarpal and metatarsal have gone into the wrist and ankle and cites for comparison the manus of *Oudenodon*, where the first digit is not reduced. This suggestion as to the fate of the first element of the first digit of the hand and foot is by no means a new one, since the same opinion was held by many of the older anatomists, but it is of interest here because of the bearing it has on the arrangement of the epiphyses, which we should expect to find in the primitive Theriodontia and Therocephalia. If specimens of these animals are ever found well enough preserved to show the epiphyses we should expect to find the arrangement exactly as it is in some lizards. There should be no epiphyses on the proximal end of the metacarpals and metatarsals, but they should be present on the distal end and on the proximal end of the phalanges.

It is to be noted from the foregoing discussion of the epiphyses of the lizards that a great many of them are of calcified cartilage. The presence of an abundance of calcified cartilage in the skeleton appears to be characteristic of the Squamata excepting, perhaps, the snakes. Dr. Williston tells me that there are always abundant evidences of calcified cartilage in the fossilized skeletons of the mosasaurs. In the ear region, on the girdle bones, on the vertebræ and, whenever preserved, the sternum and the sternal ribs, are entirely of calcified cartilage, as are also the tracheal rings. According to the same authority, this condition is never found in the plesiosaurs. There is not the slightest trace of calcified cartilage on the skeleton of these animals.

Howes and Swinnerton (13) make no mention of any epiphyses on the skeleton of the young of *Sphenodon punctatus* Gray, although they figure on Plate VI, Fig. 18, epiphyses on the foot. Albrecht, however, in 1883, called attention to certain structures on the neural spines of this form which he thought were bony epiphyses. So far as I am aware his statement has never been corroborated. I am not able to find any indications of epiphysial elements on an adult skeleton of this form, which is the only material available. The only element which might be called epiphysial is the olecranon. This is free as in the Lacertilia. Epiphyses are not wholly absent from the skeleton of the Rhynchocephalians, and it is possible that Albrecht is right. In his large work on fossil vertebrates (16) von Meyer figures a skeleton of *Saphrosaurus* on which there are quite evident epiphyses on the humeri and femora. The epiphyses are especially abundant on the lower end of the left humerus.

Just why epiphyses should develop in some forms of the reptiles and not in others is a very difficult question to answer. They are certainly not essential to the formation of good articular surfaces since they have never been observed in either the pterodactyls or the theropodous dinosaurs. These forms have a perfection of formation of the articular surfaces such as is not excelled among the Sauropsida. In the fowl there is an epiphysis at the upper end of the tibio-tarsus and a larger one on the lower end. There is also an epiphysis at the upper end of the tarso-metatarsus. The one at the upper end of the tibio-tarsus is undoubtedly a true epiphysis but the other two are more possibly elements of the tarsus which, in this case, may have an epiphysial appearance but are not true epiphyses (14). The two epiphyses at the ankle joint develop about two weeks after hatching and join with the diaphysis early. The other epiphysis is not formed until more than two months after hatching. In birds the articular surfaces are nearly as well formed as in the groups above mentioned, yet epiphyses are for the most part absent.

It can be well understood how the aquatic and semi-aquatic reptiles, such as the mosasaurs, crocodiles, turtles, plesiosaurs and their allies could have dispensed with epiphyses, but it is rather difficult to understand just why they were not developed in the Pterosauria and Theropoda, and Seeley (25) mentions epiphyses as occurring in the former.

The epiphyses which occur in the reptiles do not all have their origin in the same causes. Those on the ends of limb bones arise, undoubtedly, from different causes than do those which occur on the skull and girdle bones. Parsons calls the epiphyses at the ends of the limb bones "pressure epiphyses." This nomenclature will hold for the higher mammals where

there is a distinct pressure on the limbs but in the case of the majority of lizards, this pressure is not directly transmitted through the axis of the limb since the limbs are set, often, at angles from the body of from ten to sixty-five degrees. In the chameleons, which are to a large extent tree dwellers, the nearest approach to the mammalian attitude is attained and in these the epiphyses may be due to pressure. But in *Amblyrhynchus*, which spends a part of its time in the water, there are more epiphyses than there are in the chameleons and the limbs are set at much wider angles from the body than is the case in the chameleons. I am of the opinion that the epiphyses developed on the limbs in the majority of the Lacertilia are not due to pressure directly but that they arise from other and as yet unknown causes. It is possible that they are developed from both pressure and traction, but we need further evidence on this point.

Some of the epiphyses of the skull can be accounted for by the fact that in certain forms the elements of the skull atrophy and their remnants are left as scales of bone which resemble epiphyses, and in these cases could hardly be distinguished from epiphysial structures. Such a case of an atrophied bone is cited by Baur in the Geckonidae (15). The epiphyses which occur elsewhere on the skull and on the girdle bones may possibly be due to the strain or pull of muscles, but this is doubtful. If they do arise in this way then it must be conceded that the structures which subsequently develop into epiphyses must first be cartilage, then calcified cartilage and then bone. Whether such is the mode of origin of the "traction epiphyses" or not is still to be settled. If this should be claimed to be the case then might we not expect that in time the sternum of the Lacertilia would become osseous. As far back in geological time as we know anything about the sternum of the lizards or their relatives, it has been merely calcified cartilage. Surely the millions of years which have elapsed since the time of the early Lacertilia of the Cretaceous have given ample time for the sternum to become bony, but it is no more bony now than then. For this reason and for others it is to be doubted whether a structure can develop independently from calcified cartilage into bone although calcification is a part of the process of ossification in some forms.

The epiphyses on the carpals and tarsals of the lizards must be the degenerate elements which have become united with their fellows. This is certainly the case in some forms, but in others it is doubtful if this explanation will hold.

What the causes are which have produced epiphyses, I cannot say. It is possible that Parsons' explanation of the pull of muscles and the pressure of the body are the correct ones. So far this is merely a matter of opinion.

We have no direct evidence on these points. In this connection arises the question of the origin of the bone which goes to form any epiphysis. These and many other questions relating to epiphyses still await further investigation.

SUMMARY.

(1) There are no bony epiphyses on any of the skeletal elements of the Chelonia. (2) There are no true epiphyses on the propodials of the plesiosaurs. The conelike structures at the ends of the limb bones are in reality portions of the diaphysis and are homologous with similar elements found in all other existing groups of the Sauropsida. (3) There are no epiphyses on the skeleton of the crocodiles. (4) Epiphyses are variably present on the skeleton of the Lacertilia. (5) Epiphyses are of no importance in the classification of the larger groups of the vertebrates. (6) Parallel characters in the lizards and mammals in the presence and appearance of epiphyses on various parts of the skeleton due not to chance but to some fundamental cause. (7) The arrangement of the epiphyses on the hand and foot of lizards and mammals is of genetic importance. (8) Epiphyses are rarely present in *Sphenodon*. (9) Epiphyses are partially of a sesamoid origin but are not sesamoid bones. (10) Epiphyses are not necessary for the formation of good articular surfaces. (11) Causes of the development of the epiphyses in the Lacertilia various.

I wish in this place to express my hearty appreciation of the interest shown in my work by Drs. S. W. Williston and F. R. Lillie, and my gratitude for the help which I have received from them. The work was done under their direction and advice.

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THE CHROMOSOMES OF ANASA TRISTIS AND ANAX JUNIUS.

BY

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WITH 5 FIGURES.

ANASA TRISTIS.

The material for the study of the chromosomes in the spermatogenesis of *Anasa tristis* was obtained at Columbia, Mo., and was examined by means of sections. A careful investigation has completely confirmed Wilson's results, 05, 06, 07, respecting the number of spermatogonial chromosomes in this insect and the presence of an accessory or heterotropic chromosome, as well as the asymmetrical distribution of this chromosome, at the second maturation division. The observations here presented are, on the other hand, at absolute variance in all important points with the recently published investigation of Miss Foot and Miss Strobell, 07, who have studied and photographed smear-preparations of the testis of *Anasa tristis*.

Wherever an accurate count of the spermatogonial chromosomes can be made at full metaphase, the number has been found to be 21, as discovered by Wilson, and not 22, as originally determined by Paulmier, 99, and recently confirmed by Foot and Strobell. No exception to this result has been encountered, although the count has been made with certainty in scores of cells, and not a single case showing 22 chromosomes has ever been observed. Many of the spermatogonial groups have been drawn under high power, six of which are reproduced in Fig. 1, *A-F*, and in every instance both the number and form of the chromosomes are seen with diagrammatic distinctness, conforming in all respects with the groups as figured by Wilson. The two *m*-chromosomes and the three large ones appear in all, while one of the latter is usually bent upon itself, sometimes nearly at right angles, as in Fig. 1, *F*. It is not at all improbable that in smear-preparations this chromosome might be pulled apart at the angle, and thus account for the observations of Miss Foot and Miss Strobell. This explanation, moreover, is in a sense justified by their photographs of the spermatogonial groups, especially photos. 47

and 50, 07b, which show only two large chromosomes, while in each case two other chromosomes are lying with their ends in contact and at an angle to each other, as if they might well be portions of the large bent chromosome which has become partially severed, but which is seen intact in sections.

Side views of the spermatogonial spindles clearly prove that all of the chromosomes at metaphase lie in a flat plate and preclude the possibility, as Wilson has pointed out, that some of the chromosomes may be outside the plate at this time, and hence fail to be included in a transverse section. The spermatogonial groups, therefore, show in this material not only the same number but precisely the same form-relations of the chromosomes as described and figured by Wilson, and afford a detailed confirmation of his results.

Our sections, furthermore, lend strong support to Wilson's view that the "chromosome-nucleolus" of the growth-period is the persistent odd chromosome of the spermatogonia, and not merely a chromatin nucleolus, as maintained by Foot and Strobell. They also furnish the most indisputable evidence of the asymmetrical distribution of the heterotropic chromosome at the second division. There is not the faintest indication that it is merely a "lagging chromosome" which divides at a late period of the anaphase, as is believed to be the case by the latter observers, but, on the contrary, every stage in its passage without division to one pole of the spindle can be followed. If any doubt as to this fact should remain from an examination of side views, two of which are given in Fig. 1, *G* and *H*, it is at once removed by seeing sections of the daughter plates of the same spindle which show ten chromosomes in one and eleven in the other, as figured by Wilson. *I* and *J* of Fig. 1 represent polar views of the daughter groups of the second division, the former lacking, the latter including the odd chromosome. These two groups belong to the same spindle and were drawn from the same section at different focuses, as one was lying immediately above the other in the same cell. The odd chromosome in *J* is easily recognized at *h*; it lies just below the others, while the remaining chromosomes in the two groups correspond exactly in size and relative position. It by no means always lies on the periphery of the group, but may be found in any position. The designation "eccentric," which Miss Foot and Miss Strobell apply to this chromosome is, therefore, a misnomer, for its position outside of the group at the second division—an occurrence upon which they lay much stress—is far from being a constant one.

Several such instances as that figured in *I* and *J* have been found, and

when the two groups can be observed in the same cell, they furnish an absolute demonstration of the inequality of the distribution of the chromosomes at this division, with the result that the heterotropic chromosome passes into one of the spermatids and not into the other.

The evidence presented by a study of the chromosomes of *Anasa tristis*, obtained in a locality widely separated from the places where other observers who have examined this form have secured their material, would seem to indicate that Wilson's description, especially with regard to the behavior of the heterotropic chromosome, holds good throughout the species.

In view of the important bearing which these facts have upon the chromosome-theory of sex-determination, it has seemed advisable to add a confirmatory note to the already clear and convincing observations of Wilson on this and other hemipterans.

ANAX JUNIUS.

The spermatogenesis of this dragon-fly was first described in 1904 by Miss McGill, 04, who found a close similarity to Paulmier's account of *Anasa tristis*. Like him, she described an even number of spermatogonial chromosomes, in this case 28, and followed him in identifying the accessory chromosome of the maturation divisions with the two small chromosomes of the spermatogonia which were supposed to have united to form a bivalent at synapsis. The accessory chromosome was correctly observed to divide at the first maturation mitosis and to pass undivided to one pole at the second, as Paulmier had described for the squash-bug.

A recent study, however, of the testis of *Anax* has brought to light the fact that the same error was made as in the case of *Anasa*, since it has been determined beyond any doubt that the number of spermatogonial chromosomes is 27 and not 28. It is, furthermore, clear that the small or *m*-chromosomes of the spermatogonium divide at both maturation mitoses, and hence cannot be identified with the accessory which is one of the larger chromosomes, though not the largest of the spermatogonial group. Miss McGill was also unable to find evidence in the material which she examined of a condensed chromosome-like body persisting throughout the growth-period, but in our more recent study this has been clearly demonstrated at every stage of the resting spermatocyte until it passes on the spindle of the first maturation mitosis. Judging from its size-relations and other considerations, it is unquestionably the odd or heterotropic chromosome.

Our re-examination of *Anax*, therefore, brings this dragon-fly into complete accord with those insects, like *Anasa* and *Protenor*, in which an odd number of chromosomes is found in the spermatogonia and an asymmetrical distribution of the heterotropic chromosome takes place at one of the maturation divisions, resulting in a dimorphism of the spermatids. These observations consequently lend additional support to Wilson's interpretation of the relation of the chromosomes to sex-production.

In the following account, the general course of spermatogenesis in *Anax* will not be traced, as this will be found in Miss McGill's original paper, but only such points will be brought out as require correction or further amplification.

THE SPERMATOGONIAL GROUP OF CHROMOSOMES.—As already stated, the number of chromosomes is 27. This has been determined with perfect clearness in a very large number of cases and has been found to hold true without exception. Whenever the equatorial plate is cut transversely at full metaphase, the chromosomes are seen lying well apart and sharply defined, while the counting of their number is a matter of the greatest ease. It is only in unfavorable cases, as in insufficiently extracted sections or through a close crowding of the chromosomes, that an accurate count is rendered uncertain. In properly stained sections, an unlimited number of cells have been found which show 27 distinct chromosomes, never more.

The size differences in the chromosomes are well marked, although not so strikingly as in many of the Hemiptera. Between the *m*-chromosomes, which are the smallest pair, and the largest pair or macro-chromosomes, which are usually quite perceptibly larger than any of the others, the remaining chromosomes, except the odd one, may be arranged in pairs of intermediate sizes. The differences among the latter, however, are only slight and some pairs are of approximately equal size (Fig. 2, *A* and *B*). After pairing off all the others, one will be left without a mate, and this will be found among those of intermediate size. The exact chromosome cannot be identified as the odd one, as the sizes are not sufficiently differentiated to allow of an accurate pairing of all the remaining chromosomes, and furthermore some of the chromosomes exhibit a certain irregularity of form which increases the difficulty.

In side views, the spermatogonial spindles show a flat equatorial plate at metaphase, with all of the chromosomes lying in the same plane (Fig. 2, *C*), and here, as in *Anasa*, polar views at this stage exhibit without doubt the full number of chromosomes.

The spermatogonial divisions have been adequately described by Miss

McGill, and the details need not be repeated here. All of the chromosomes divide longitudinally and pass in similar daughter groups to the poles, while after the last division the young spermatocytes enter upon the growth period.

THE FEMALE SOMATIC CHROMOSOMES.—An attempt has been made to determine the number of chromosomes in the female germ-cells, and sections of the ovaries of the nymphs have been carefully examined. Although no oögonial divisions have been encountered, possibly because we have not been able to obtain ovaries from young enough nymphs, nevertheless mitosis occurs abundantly among the follicle cells and a detailed search has disclosed several cases where the sections were favorable for counting the chromosomes. It might be remarked that Miss McGill, 06, in her study of the ovarian history of the germ-cells in the same dragon-fly has shown that the follicle cells have the same origin as the oögonia, both being differentiated from cells of the end-filaments of the egg-strings, and there is, therefore, every reason for supposing that their chromosomes are essentially the same. The number of chromosomes in the follicle cells has been determined in several instances, and wherever an accurate count has been possible, it has been found to be 28. Two of these cases are drawn in Fig. 2, *D* and *E*, and show the full number distinctly. The size differences are not as well marked as in the male groups, although the *m*-chromosomes are readily identified in *D*, and a decidedly largest pair is seen in *E*. The chromosomes, however, appear somewhat smaller and show a slightly greater irregularity of form than do those of the male group. This is especially true of *D*, the group *E* being more nearly like the male in the size and shape of the chromosomes. In both of these cases, and also in a few others where an accurate count has been possible, the chromosomes had not quite come to full metaphase. They had, therefore, not reached their complete condensation and were evidently not lying in their final orientation on the spindle, with the result that they appeared in the sections at varying angles. This fact explains their irregularity of form, and, doubtless, a longer search would have revealed more favorable cases showing a closer similarity to the size and shape of the male chromosomes. Still, the cases illustrated exhibit a striking correspondence, and it is a matter of great importance for the theory of sex-chromosomes to know that the female number is 28, while that of the male is 27. It is, furthermore, of interest to find that the same differences among the chromosomes exist in a member of the Odonata as have been shown to occur in the Hemiptera and other groups of insects.

THE GROWTH PERIOD.—The growth period has not been followed throughout in all the detail that is desirable, and the important question of the character of the synapsis must for the present be left undecided. Preceding the contraction-phase a stage is found where the chromatin appears in the form of fine threads scattered throughout the nucleus or more or less massed in loose tangles. Some of these strands are V or Y-shaped, or are seen lying in separate pairs or as irregular crosses (Fig. 2, *F*.) The appearance might readily be interpreted as the result of a longitudinal conjugation of univalent chromosomes which are now opening out to form elongated loops or ribbons. In view of the work of v. Winiwarter, 00, the Schreiners, 04-06, Lerat, 05, Janssens, 05, Bonnevie, 05-07, Stevens, 05, 06, and others, who have brought forward evidence in support of parallel conjugation, it is entirely possible that a more careful study of this and earlier stages in the growth-period of *Anax* might throw light upon the true nature of synapsis in this insect, but at present the evidence is insufficient for a demonstration.

The odd chromosome, as a dense nucleolus-like body, is quite conspicuous at this stage, staining deeply in both hæmatoxylin and safranin, and is frequently found to be constricted into a dumb-bell shape (Fig. 2, *F*, *h*). It does not occupy a constant position in the nucleus, sometimes lying near the membrane, at other times at any point within the nuclear area. Often it is seen close against the plasmosome, while as often it may lie at quite a distance from it.

This stage is followed by the contraction-phase (Fig. 2, *G*), in which, however, the chromosomes never become condensed into a compact mass. The threads now appear much thicker and longer than at an earlier period and are usually arranged in long, loose loops, the ends of which are more or less directed toward one pole of the nucleus, constituting the so-called "bouquet-stage." Curiously enough, the longest and thickest ribbon, which is the one that will give rise to the largest tetrad later on, frequently lies outside the "bouquet" and is seen stretching half-way around the nucleus close against the membrane, as shown in Fig. 2, *G*. This elongated chromosome is often interrupted at its middle point, as are, in fact, some of the looped threads, which consequently exhibit a bivalent nature. The break, moreover, is found to persist from now on to the time when the final condensation of the chromosomes takes place in the formation of the crosses. It may represent, of course, the line of suture between two univalent chromosomes conjugating end to end, or it may be equally well interpreted as the point of contact between the

ends of two chromosomes which have previously undergone a parallel conjugation and subsequently opened out to form a loop or ribbon.

During the bouquet-stage, the chromosome-nucleolus, which we may speak of as the heterotropic chromosome, as it directly passes into this body without ever assuming a thread-like form, is always present and is frequently constricted in the middle or even completely divided into halves. (Fig. 2, *H*).

It is not until after the bouquet-stage is passed and the chromosomes, in the form of bivalent threads of varying length, have scattered through the nucleus, that the longitudinal split becomes visible, while the interruption at the middle of each thread becomes very conspicuous (Fig. 2, *H* and *I*). Delicate strands of linin may be seen bridging across the transverse split and connecting the halves with each other. One of the double ribbons is always much longer than the rest and can be traced continuously back to the long chromosome of the bouquet-stage and forward to the largest tetrad.

The heterotropic chromosome is still seen to be double, although it has lost its dumb-bell form and is now spherical with a distinct line of cleavage dividing it into halves, as if the two rounded portions of the dumb-bell had flattened down upon each other (*H* and *I*).

Late in the growth period and preparatory to the first maturation division, the longitudinally split threads showing their quadripartite character, as seen in the last figure, begin the process of condensation. The longitudinal halves fuse completely and become much shorter and thicker, although the transverse interruption is still present as a clearly marked constriction at the middle point of the chromosomes. This stage is drawn in Fig. 2, *J*, in which the large bivalent is quite easily recognized.

The odd chromosome can be followed continuously from the earliest stages of the growth-period, until it appears as shown at *h* in this figure. The two hemispheres have become rounded out and slightly drawn apart, although not completely separated, and in this condition it is taken up on the spindle which is formed a little later.

The thick bivalent rods next undergo a still further condensation and are converted into crosses by outgrowth of transverse arms at the level of the constriction. These arms, however, do not extend so far as to confuse the original longitudinal axis, a condition which is especially conspicuous in the largest cross where the transverse and longitudinal arms remain quite unequal, although it is more or less pronounced in all. As this inequality in the length of the arms is retained until the crosses are taken up on the spindle, an identification of the long axis of the cross with that

of the threads of the growth-period is made certain, and, furthermore, it is evident that the crosses are placed lengthwise upon the spindle.

The number of chromosomes is now easily observed, fourteen in all, thirteen of which are in the form of crosses, while one, the heterotropic, is a simple, condensed, bipartite body. As the crosses condense, their centers, just before the disappearance of the nuclear membrane, become lighter and soon take the stain only faintly, while by the time the spindle is visible they have become completely hollowed out. The series of changes taking place in the formation of the crosses, and their conversion into the open tetrads may be followed through Fig. 2, *J-L*, and Fig. 3, *A* and *B*, successively. These figures also demonstrate the relation of the long axis of the cross to that of the spindle which are seen to be parallel.

THE FIRST MATURATION DIVISION.—After being taken up on the spindle the open tetrads rapidly undergo a condensation; all traces of the space between the arms disappear, and by the time the prophase is ended they have the form shown in side view in Fig. 3, *C*. Although the quadripartite character of the thirteen tetrads is still indicated by slight depressions or grooves on the surface, there is at this stage no separation of the four elements which have become fused with each other. In side views the broad face of the tetrads shows the demarcation of the four compound parts, while those tetrads which are seen on edge in these views appear as dumb-bell shaped bodies and therefore cannot be distinguished, except in some cases by size, from the heterotropic chromosome which has this appearance when seen in either vertical plane. In order to determine with certainty which chromosome is the heterotropic, one must examine the metaphase groups in polar view, in which all of the tetrads appear as dumb-bell shaped bodies, while the heterotropic is seen as a single chromosome, since its constriction lies in the horizontal plane. Fig. 3, *D*, shows these relations very plainly; there are thirteen double chromosomes which are the thirteen tetrads seen in polar view, while the heterotropic at *h* appears as a single oval body. The range in size from the smallest to the largest tetrad is also clearly indicated. *E* is another polar view, but at a slightly later time when the separation of elements is just beginning in four of the tetrads. This breaking apart of the component parts of the tetrad proceeds rapidly with the beginning of the anaphase, until all four elements are entirely distinct from each other as the opposite pairs advance toward the poles of the spindle (*F* and *H* of Fig. 3). *G* shows a daughter group in the early anaphase before the members of all of the pairs are entirely separated, while *I* is a similar view a little later. In the latter, there is no longer any connection be-

tween the halves of the dyads, which, however, are still lying side by side. In both drawings the heterotropic is recognized at a glance, as it is the **only** chromosome in the group without a mate.

The **divergence** of the chromosomes occurs at unequal rates, those on the periphery lagging **behind**, those in the center taking the lead, while the heterotropic lags most of all and **in** late anaphases, when the others are grouped about the poles in a dome-shaped **mass**, the halves of the heterotropic may usually be seen some distance behind in the process (*J* and *K* of Fig. 3).

As the chromosomes reach the poles, they become closely crowded together and partially break up into a reticulum, although to a certain extent their identity is still retained in the nodes of the network. The centrosomes fade out and a new nuclear membrane forms but only to disappear shortly afterwards in preparation for the second division. In fact, there are indications that in some cases at all events this intervening resting stage between the two divisions is practically omitted.

THE SECOND MATURATION DIVISION.—As soon as the nuclear membrane fades away the chromosomes reappear as dumb-bell shaped bodies, and as the new spindle forms they take up their position in the equatorial plate with the constriction at right angles to the long axis of the spindle. There are thirteen of these dyads, and the one single chromosome, the heterotropic. Even before the other chromosomes pass definitely into the equatorial plate, the heterotropic without undergoing division begins usually to move in advance toward one of the poles, as may be seen in Fig. 4, *A* and *B*, and it has nearly completed its journey before the divergence of the other chromosomes is commenced. There is, however, a good deal of irregularity in the movement apart of the chromosomes, especially during the early anaphase, the halves of each pair seeming to diverge at a more or less independent rate. This independence of movement, not only of the heterotropic, but of the other chromosomes as well, increases the difficulty of mechanical or electrical theories of the cause of divergence.

Early stages in the progress of the undivided heterotropic chromosome toward one pole are shown in *A* and *B* of Fig. 4, while still later stages are represented in *C* and *D*. In the former drawings, both the *m*-chromosome and the macro-chromosome are distinctly recognized in side view, while in *D*, although the heterotropic is still visible at one pole, the other chromosomes are closely massed together. As in *Anasa tristis*, polar views at the anaphase of this division clearly demonstrate the dimorphism of the daughter groups, since thirteen single chromosomes appear in one and fourteen in the other, the former being the one that lacks the hetero-

tropic. At about the stage of the anaphase represented in *C*, this inequality in the number of chromosomes composing the daughter plates has been determined in several well marked cases where the groups have been observed in polar view at different focuses on one and the same spindle. *E* and *F* are such anaphase groups drawn from the same section, *E* including the heterotropic which is seen at *h*, *F* lacking this chromosome. In each group the *m*-chromosome and the macro-chromosome are readily picked out, while the two groups, if added together, would exactly reproduce the conditions seen in the spermatogonial plate.

It has been said that the heterotropic chromosome *usually* passes in advance of the others during the anaphase of the second division, as in the majority of cases, where this stage is observed in side view, such a condition is quite evident. But, strangely enough, a few cells are always encountered, often in the same cyst with the others, which show the heterotropic lagging behind instead of preceding the other chromosomes, as in the case of *Anasa*. Of course it cannot be determined with certainty that the lagging one is the same chromosome as the precocious heterotropic of the commoner cases, but there can be little doubt that the two are identical. This conclusion is rendered very probable from their similarity in size and also from the fact that when the heterotropic is seen in advance a lagging chromosome is not observed, and vice versa. It is, therefore, to be supposed that the heterotropic may pass indifferently either in front of the other chromosomes or behind them.

SOME GENERAL CONSIDERATIONS.—As a result of our re-investigation of the spermatogenesis of *Anax junius*, it has been established beyond doubt that this dragon-fly, in the behavior of the chromosomes of the male germ-cells, closely parallels the conditions which have been observed in some of the Hemiptera and other higher groups of insects. In the differentiation of its chromosomes as *m*-chromosomes, macro-chromosomes, and chromosomes of intermediate sizes; in the occurrence of an odd number of chromosomes (27) in the male groups and of this number plus one (28) in the female groups; in the presence of an accessory or heterotropic chromosome which persists as a condensed body throughout the growth-period and passes undivided at the second maturation division into one of the spermatids, a strict parallelism may be recognized between *Anax* and those other insects, of which *Anasa tristis* may be taken as a type, which possess a heterotropic chromosome. In at least one of the Odonata, therefore, a dimorphism of the spermatozoa occurs, and the theory of the determination of sex by differentiated sex-chromosomes receives additional support from this group of insects.

Our observations, however, throw no light upon the important problem of reduction, yet certain conditions in the formation of the tetrads and the distribution of their component elements may be briefly indicated. A slightly schematic representation of the formation of the tetrads, showing also the axial relations of the longitudinally split bivalent threads of the growth-period and of the tetrads on the spindle is given in Fig. 5. In each of the successive figures from *A* to *I*, it is the largest chromosome that is shown, while all are drawn under the same magnification. Many of the chromosomes in this diagram are redrawn with only unimportant modification from the macro-chromosome already represented in previous figures. For example, *D* is taken from Fig. 2, *K*; *H*, from Fig. 3, *F*; etc.

It has already been pointed out that the long axis of the tetrad is identical with the long axis of the chromatin threads of the growth period, and since the figures have clearly shown that the tetrads are placed upon the spindle lengthwise, it follows that the first maturation division must separate univalent chromosomes and be therefore a reducing division on the assumption that an end to end conjugation takes place between individual chromosomes at synapsis. And continuing this assumption, the second division must be equational, since the chromosomes are oriented in such a way as to distribute the halves of the dyads which are separated by the longitudinal split. This conclusion was drawn by Miss McGill in her original paper. If, however, it should prove true of this form that a parallel conjugation occurs, as has been suggested, the first division would still be a reducing one, since the axes of the crosses are not reversed by the drawing out of the transverse arms and the attachment to the spindle fibers is at the end of the longitudinal arms, as seen in Fig. 5, *E*. But in the event of the chromosomes conjugating side to side, it would still have to be shown that they separate after fusion as the originally distinct chromosomes without loss of identity before a real reduction could be established. The crucial problem, then, in the whole question of reduction lies at this point, and until further light is thrown upon it by future investigation, it is futile to indicate possibilities and frame speculations.

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NOTE.—All of the drawings were made as carefully as possible with the camera under a 2 mm. Zeiss apochromatic and compensation-ocular 12. They were then enlarged with a drawing camera two and a half times, corrected by comparison with the objects, and reduced by one-third in reproduction. Their final magnification as they appear here is about 3583 diameters. Although the chromosomes are represented as accurately as possible, and are in no sense schematized, an attempt has not been made to show the details of the achromatic structures.

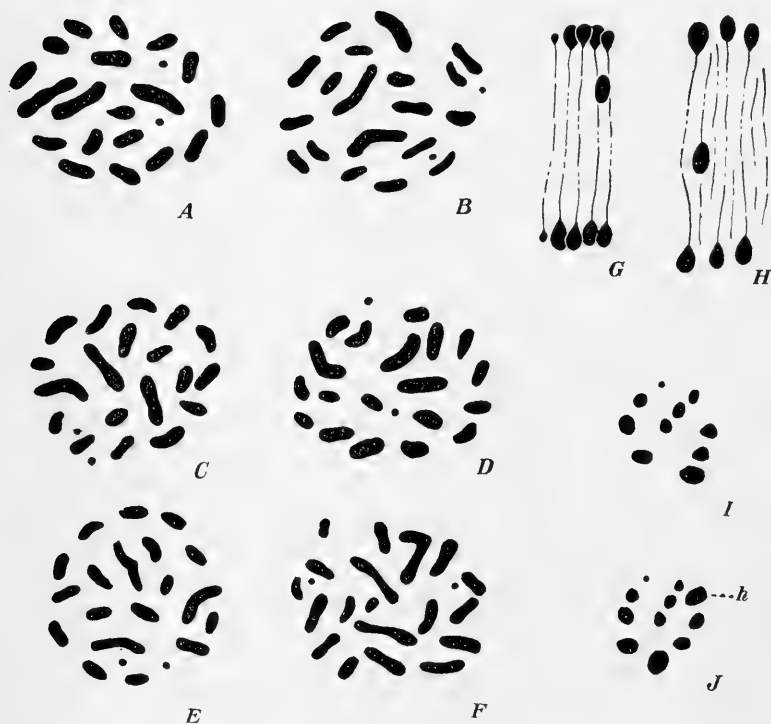


FIG. 1. *Anasa tristis*. *A-F*, spermatogonial groups; *G, H*, anaphases of second division, showing division of *m*-chromosomes in *G* and the undivided heterotropic chromosome on both spindles; *I, J*, anaphase groups from the same spindle, polar view, second division, showing *m*-chromosome and macrochromosome in each and the heterotropic (*h*) in *J*.

FIG. 2. *Anax junius*. *A, B*, Spermatogonial groups; *C*, metaphase of spermatogonial division; *D, E*, chromosome-groups from follicle cells of ovary; *F*, early stage of growth-period of the male, showing heterotropic (*h*) and plasmosome (*p*); *G*, contraction-phase, "bouquet-stage," showing the bivalent macro-chromosome; *H, I*, later growth-period, showing longitudinal splitting of chromosomes and the divided heterotropic (*h*); *J, K, L*, successive stages of the spermatocyte-nucleus, showing condensation of the bivalent chromosomes to form crosses.

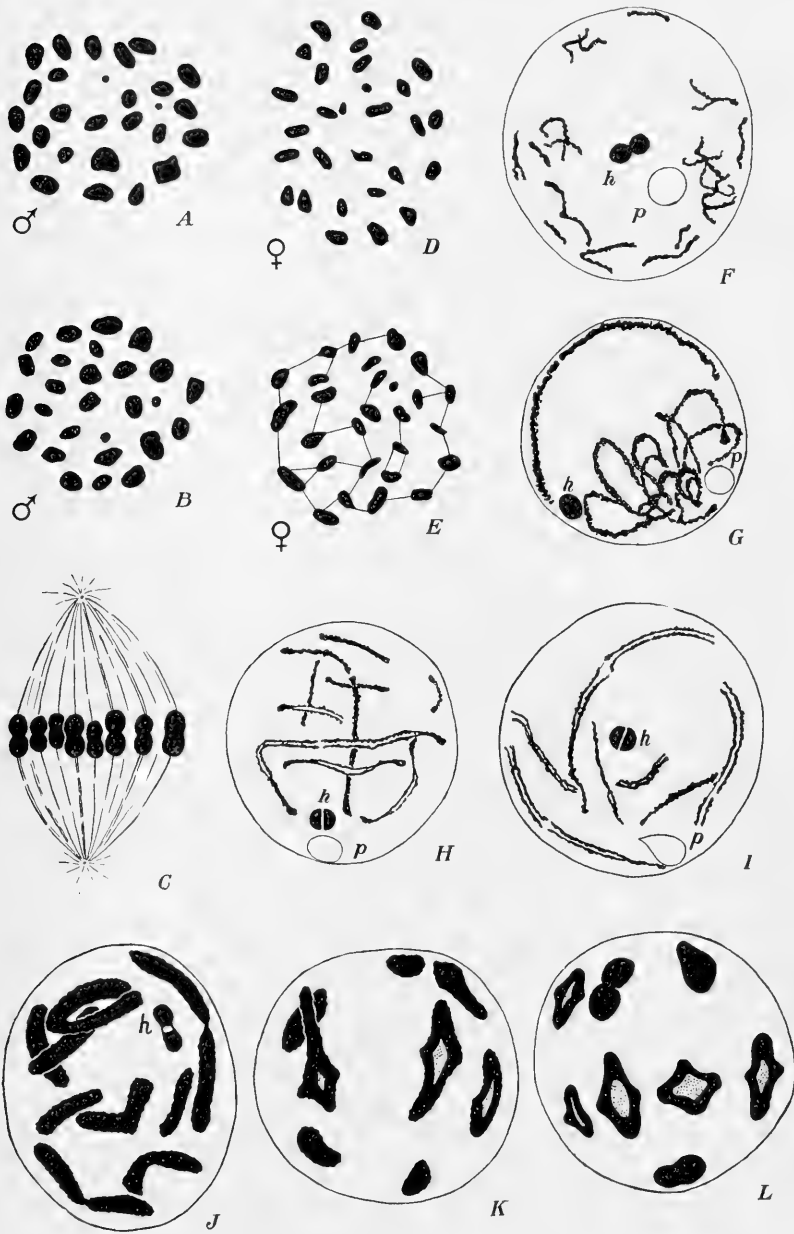


FIG. 2.

FIG. 3. *Anax junius*. *A, B*, Late prophases of first division, showing in some cases the four parts of the crosses; *C*, metaphase, first division, showing condensed tetrads; *D, E*, metaphase groups, polar view, showing the 13 tetrads and the heterotropic (*h*) in each case; *F*, beginning anaphase, first division, showing complete separation of the parts of the tetrads; *G*, early anaphase group, polar view, showing 13 dyads and the single heterotropic; *H*, early anaphase, first division; *I*, anaphase group, polar view, same stage as last; *J, K*, later anaphases, first division, showing the divided heterotropic lagging.

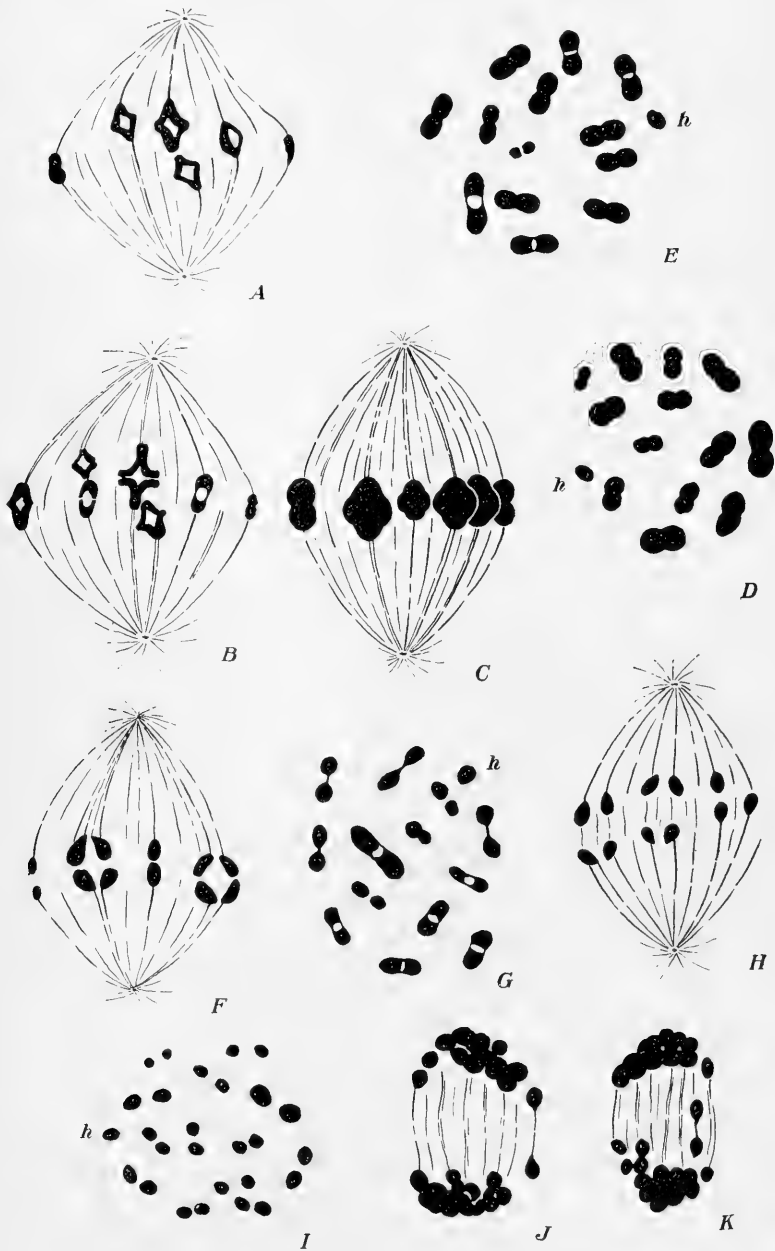


FIG. 3.

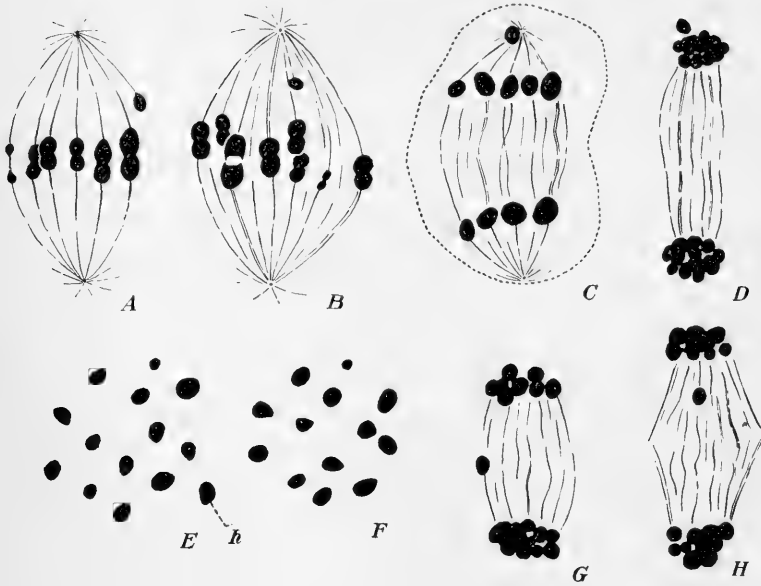


FIG. 4. *Anax junius*. A, B. Second spermatocyte-division, showing undivided heterotropic passing to one pole and the *m*-chromosome dividing in A; C, D, later anaphases, second division, still showing undivided heterotropic in advance of other chromosomes; E, F, anaphase groups from same spindle, polar view, second division, showing the *m*-chromosome and macro-chromosome in each and the heterotropic (*h*) in E; G, H, late anaphases, second division, showing the heterotropic lagging behind instead of advancing in front of the other chromosomes.

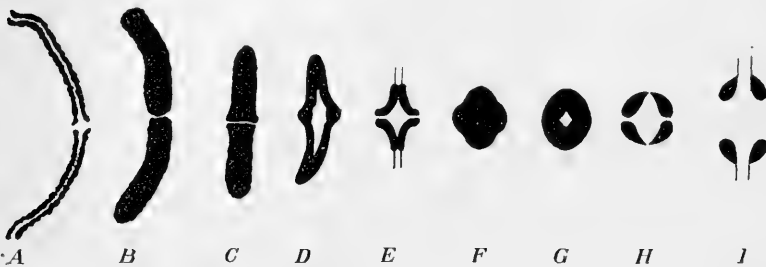


FIG. 5. *Anax junius*. Slightly schematic representation of successive stages in formation of tetrads and axial relations of same; A, longitudinally split bivalent from late growth-period; B, C, condensation of bivalents; D, formation of cross; E, cross with the four parts distinct, oriented on spindle with original long axis placed lengthwise; F, condensation of tetrad; G, tetrad beginning to open; H, tetrad completely separated into 4 parts; I, divergence of the divided dyads in anaphase of first maturation mitosis.

MODELS OF THE PANCREAS IN EMBRYOS OF THE PIG,
RABBIT, CAT, AND MAN.

BY

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WITH 6 TEXT FIGURES.

The pancreas of mammals is now generally described as arising in the embryo from a dorsal pancreas, and a ventral pancreas, the latter often being subdivided into a right and a left part. Each embryonic portion has its own duct. The duct of the ventral pancreas has been known as the duct of Wirsung, for which term the Basle nomenclature substitutes "pancreatic duct" (*ductus pancreaticus*). The duct of the dorsal pancreas, formerly known as the duct of Santorini, which becomes secondary or disappears in man, is called the "accessory pancreatic duct" (*ductus pancreaticus accessorius*). The elimination of personal names is desirable, but the terms substituted may be criticised as applicable only to man. The duct of the dorsal pancreas which alone persists in the pig cannot properly be called either the pancreatic or the accessory pancreatic duct.

Appreciating this difficulty, Revell, ⁰², proposed the terms *ductus hepatopancreatis seu dorsopancreatis*, and *ductus ventropancreatis*. The compound terms here introduced are contrary to the principles of the Basle nomenclature and are not in good Latin form. The following terms avoid these difficulties; they accord with the Basle system, and can be used both in human and comparative anatomy.

LATIN.

ENGLISH.

Ductus pancreatis dorsalis = Duct of the dorsal pancreas.

Ductus pancreatis ventralis = Duct of the ventral pancreas.

These terms will be used in the following pages. Where no emphasis upon morphological or embryological relations is desired, the name pancreatic duct is often sufficient.

¹This investigation has been aided by a Bullard Fellowship, established in memory of John Ware.

PIG EMBRYOS.

Wlassow, 95, was the first to describe the development of the pancreas in the pig. He figures a model and transverse sections of the pancreas in an 8.7-mm. pig, and also describes with the aid of transverse sections a still younger embryo (8 mm.).

Völker, 02, describes the development of the pancreas of the pig, from a series of models in the embryological collection of the University in Prague. He pictures three of these in Figs. 19, 20, and 21, but unfortunately does not give either the age or length of the embryos from which the models were made.

Lewis, 03, reconstructed a 12-mm. pig embryo, and in Plate III shows incidentally the gross relations of the pancreas.

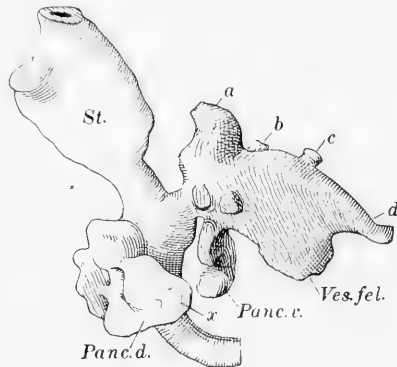


FIG. 1. Reconstruction from a pig embryo of 5.5 mm. (H. E. C. 915). $\times 55$ diams. *a, b, c, d*, cords of hepatic cells. *Panc. d.*, pancreas dorsale. *Panc. v.*, pancreas ventrale. *St.*, stomach. *Ves. fel.*, vesica fellea. *x*, ventral process of the dorsal pancreas, on the right of the portal vein.

The first model which I shall describe is from a pig embryo 5.5 mm. in length (Harvard Embryological Collection, Series 915). In Fig. 1 it is drawn from the right side, as are the models of Wlassow and Völker.

A little beyond the stomach (*St.*) the hepatic diverticulum opens into the ventral side of the duodenum. Distally it presents a considerable enlargement from which project several cords of hepatic cells (*a, b, c, d*, etc.), one of which will ultimately form the hepatic duct. The gall bladder (*Ves. fel.*) at this stage is represented by a distinct pouch from the distal, posterior or caudal wall of the diverticulum. Another outgrowth from the posterior wall of the diverticulum is found near its intestinal orifice; this is the ventral pancreas (*Panc. v.*). It is a flattened triangular structure attached to the hepatic diverticulum by a stalk

which has no lumen. The expanded basal portion of the triangle, situated in the ventral mesentery, extends laterally to both sides, but chiefly to the right in an obliquely dorsal direction. It is closely applied to the ventral wall of the right vitelline vein.

The dorsal pancreas (*Panc. d.*) is an outgrowth from the dorsal wall of the intestine nearly opposite the orifice of the hepatic diverticulum. It

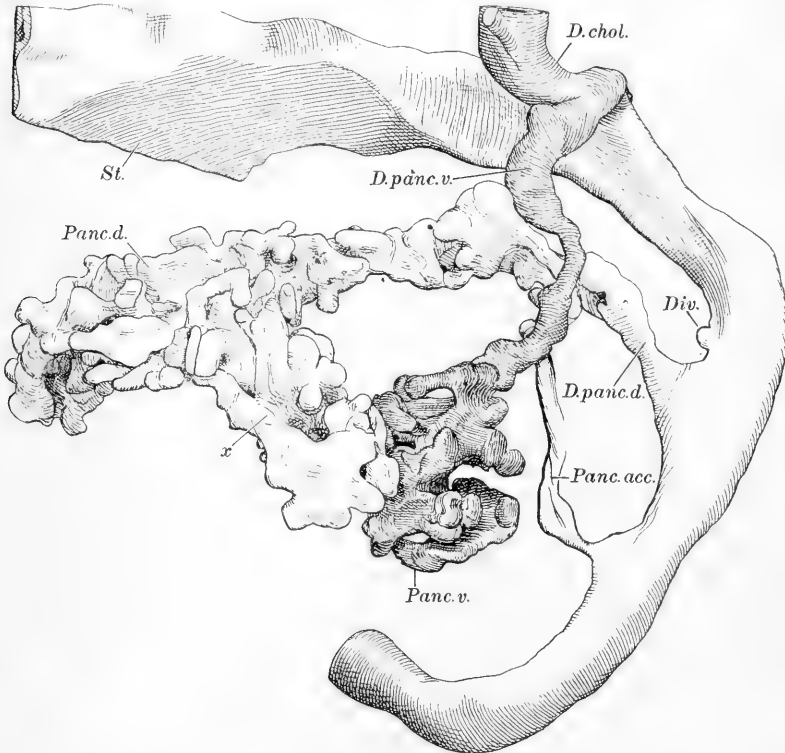


FIG. 2. Reconstruction from a pig embryo of 20 mm. (H. E. C. 60). $\times 55$ diams. *Div.*, duodenal diverticulum. *D. chol.*, ductus choledochus. *D. panc. d.*, ductus pancreatis dorsalis. *D. panc. v.*, ductus pancreatis ventralis. *Panc. acc.*, pancreas accessorium. *Panc. d.*, pancreas dorsale. *Panc. v.*, pancreas ventrale. *St.*, stomach. *x*, ventral process of the dorsal pancreas on the right of the portal vein.

is already of greater dimensions than the ventral pancreas. It extends dorsally in the great omentum, and sends a subdivision (*x*) to the right and ventrally, reaching the dorsal wall of the vitelline vein. The vein is thus being encircled on its right side by the two parts of the developing pancreas (*Panc. d.* and *v.*).

The second model (Fig. 2) represents the pancreas in an embryo of 20 mm. (H. E. C. 60). It is figured somewhat ventrally from the right side. It represents a much more advanced condition of the pancreas than that seen in Lewis' reconstruction of the 12-mm. pig embryo, but corresponds in many ways to Völker's description of embryo XIII (02, p. 87).

The common bile duct (*D. chol.*) enters the intestine a little beyond the pyloric end of the stomach (*St.*). The duct of the ventral pancreas (*D. panc. v.*) will be observed to have the same relation to the common bile duct as already shown in Fig. 1. As in the earlier stage the distal end of the ventral pancreas is in relation with a subdivision (*x*) of the dorsal pancreas. Although the relation has become more intimate an actual anastomosis on the right side of the vitelline vein has not yet occurred. The great change in relations in Fig. 2, as compared with Fig. 1, has accompanied the development of the duodenal loop of the intestine.

The portion of the intestine to which the duct of the dorsal pancreas is attached has been carried ventrally, and the duct of the dorsal pancreas now passes across the ventral pancreas on the median side of the latter. The two portions of the pancreas are in very close relation at their place of crossing, but in this embryo anastomosis between them has not occurred. Later, according to Völker, the dorsal pancreas fuses with the ventral at this point. This fusion sometimes takes place sooner than the anastomosis through the process (*x*) on the right of the portal vein; sometimes later. By the crossing of the dorsal pancreas with the ventral, the portal vein becomes surrounded by a ring of pancreatic tissue, the "anneau periveneux" of authors.

Stoss' observation, 91, that in the adult pig only the duct of the dorsal pancreas persists, receives confirmation from the investigations of Wlasow and Völker. Both found embryological evidence that after the dorsal pancreas had anastomosed with the ventral, the connection with the common bile duct atrophied, leaving only one pancreatic duct, that of the dorsal pancreas.

The outlet of the duct of the ventral pancreas has become obliterated in pigs of 24 mm. as seen in the Harvard Collection. The position of the remainder of this duct is indicated in the adult pig by a lobe of pancreas which extends nearly to the bile duct. The pancreatic ring about the portal vein is retained. Two lobes extend distally from the ring, corresponding with the dorsal and the ventral pancreas of Fig. 2.

An interesting feature of the model of the 20-mm. pig (Fig. 2) is the presence of an accessory pancreas (*Pan. acc.*). It arises from the dorsal

wall of the duodenum, a little beyond the duct of the dorsal pancreas, and in line with it. Thus it is a dorsal structure. Where the duct of the accessory pancreas passes through the intestinal musculature it is somewhat constricted, suggesting that eventually it might become separated from the intestinal epithelium. This outgrowth has a distinct lumen and is somewhat expanded at its distal extremity where the cells are of the same nature as those in the other portions of the pancreas. So far as known such an accessory pancreas in an embryo has not been figured previously. This diverticulum and the small "accessory pancreas" pictured by Völker (p. 86, Fig. 21) will be discussed in the following paper.

Another unusual condition of the pancreas was found in a 12-mm. pig embryo where a process of the ventral pancreas extended to the left, passing around the ventral side of the duodenum and nearly reaching the dorsal pancreas; thus the duodenum was surrounded for more than three-fourths of its circumference by pancreatic tissue. This condition suggests how a ring of pancreas surrounding the intestine may develop embryologically. Although I am not aware that such a ring has ever been found in the adult pig, Ecker, 62, observed a case in man where the head of the pancreas completely encircled the descending part of the duodenum. This is a rare occurrence. Apparently only one other similar case has been observed (Symington, 85).

RABBIT EMBRYOS.

Hammar, 93, in his work upon the development of the liver incidentally figured the condition of the pancreas, as found in rabbit embryos of 3 mm. (10 days), 4.5 mm., 5 mm. (11 days), and 8 mm. (Fig. 1, 2, 3, u. 4, Taf. XI); and in a later paper, 97, he adds a figure of the pancreas of a rabbit of 10 mm.

Joubin, 95, describes the pancreas as found in rabbit embryos of 13, 14, 15, 18, and 21 days.

Brachet, 96, has modeled and described the pancreas in embryos of $10\frac{1}{2}$, $11\frac{1}{2}$, $12\frac{1}{2}$, and $13\frac{1}{2}$ days (Plate XVIII, Figs. 4, 5-6, 7, and 8).

Helly, 01, has modeled the pancreas in embryos of 3.8, 4.8, 5.4, and 7 mm. (Fig. 1-2, 4, 5, u. 6, Taf. XV). He also investigated embryos of $7\frac{1}{2}$ mm., 8 mm., and $3\frac{1}{2}$ cm. His conclusions agree essentially with those of Brachet.

The model which I shall describe is from a rabbit embryo of 14 days (11 mm.). It shows a more advanced condition of the embryonic pan-

creas than has been modeled heretofore in the rabbit. In the drawing (Fig. 4) the model is viewed somewhat ventrally from the right side. The stomach (*St.*) has revolved to the left, so that the pylorus extends towards the right side of the embryo. The duodenum extends from the pylorus transversely across the median line to the right, then descends for a long distance on the right side of the portal vein, ventral to the right Wolffian body. It then turns back on the median side of its descending course. Slightly to the right of the median line at the top of the long descending branch of the duodenum, the bile duct (*D. chol.*)

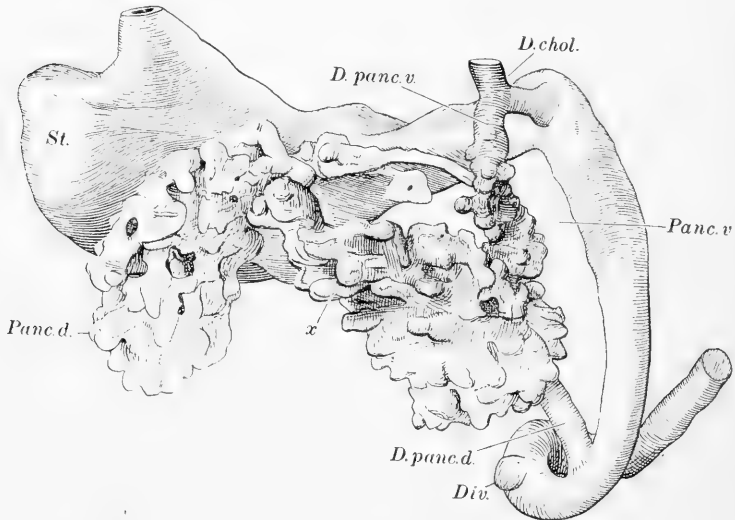


FIG. 3: Reconstruction from a rabbit embryo of 14 days, 11 mm. (H. E. C. 1327). $\times 55$ diamis. The lettering is the same as in Fig. 2.

joins the intestine. Entering the bile duct quite close to its intestinal orifice, we see the duct of the ventral pancreas (*D. panc. v.*). By comparing Figs. 2 and 3 we see that essentially the same process of development has occurred in the rabbit as in the pig. Simultaneously with the formation of the duodenal loop, the opening of the duct of the dorsal pancreas (*D. panc. d.*) has been carried a long distance beyond the opening of the common bile duct.

The ventral pancreas (*Panc. v.*) extends across the proximal part of the dorsal pancreas (*Panc. d.*), the two having anastomosed at the place of crossing. From the investigations of Schirmer, 93, and Joubin, 95, it would appear that an anastomosis at this place does not occur invari-

ably, as both have found cases in the adult rabbit where the supposed ventral pancreas was entirely free from the dorsal.

In the 11 mm. embryo, a part of the dorsal pancreas beyond the place of anastomosis is represented only by a small strand which passes on the left of the portal vein, and connects with the main portion of the dorsal pancreas (*Panc. d.*), which lies in the mesogastrium.

Thus the portal vein in the rabbit as in the pig is surrounded by a ring of pancreatic tissue, as was observed by Brachet and Helly.

The pancreas in the adult rabbit has been investigated by Bernard, 56, Schirmer, 93, Joubin, 95, Brachet, 96, and others. The duct of the dorsal pancreas occurs regularly. Bernard and Joubin found several cases where both ducts were present; Schirmer found 7 cases out of the 22 specimens examined, where both occurred; and Brachet believes that both ducts generally persist.

CAT EMBRYOS.

Felix, 92, was the first to study the pancreas in the embryo cat. In the adult he knew of only one pancreatic duct, that of the ventral pancreas. In his oldest cat embryo (11 mm.), he thought that the dorsal pancreas was degenerating, hence he concluded that only the ventral pancreas is represented in the adult.

Hammar, 93, in connection with his work upon the development of the liver, figures a model from a cat embryo 5 mm. long (Fig. 5, Taf. XI). It shows the dorsal pancreas arising from the dorsal wall of the intestine nearly opposite the opening of the common bile duct.

Helly, 91, demonstrated the error of Felix's conclusion, since he found in an embryo of 10 mm. that the dorsal pancreas was much larger than the ventral, and showed no trace of degeneration.

The model which I am about to describe is from an embryo 10.7 mm. in length (H. E. C. 474). It is represented (Fig. 3) somewhat ventrally from the right. The stomach (*St.*) has swung over to the left side of the embryo, its pyloric end now extending toward the right. The duodenum, proceeding from the pylorus, extends also toward the right and anteriorly, crosses the median line, then bending posteriorly, descends for some distance. Near this bend the bile duct (*D. chol.*) enters the intestine.

The ventral pancreas (*Panc. v.*), situated in the lesser omentum, is considerably smaller than the dorsal outgrowth. It is entirely free from the latter, although the two are in close proximity ventral to the portal

vein. Its duct (*D. panc. v.*) extends through the ventral mesentery to enter the bile duct near its intestinal orifice.

The dorsal pancreas (*Panc. d.*) ventral to the portal vein, lies in the dorsal mesentery and extends through it into the mesogastrium. Its duct (*D. panc. d.*) passes through the dorsal mesentery to enter the duodenum a little beyond the common bile duct. The dorsal pancreas has developed a short process (*x*) which we can compare with the subdivision *x* in the pig (Fig. 2). That such a process develops in the cat we know from the description and figures of Heuer, 06. He says (p. 107) :

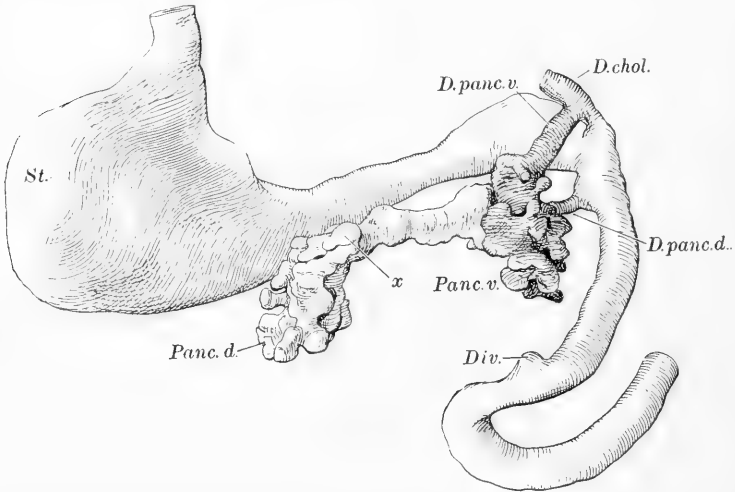


FIG. 4. Reconstruction from a cat embryo of 10.7 mm. (H. E. C. 474). $\times 55$ diams. The lettering is the same as in Fig. 2.

Lying in the plica duodeno-jejunalis between the caput and cauda is a narrow strip of glandular tissue, the shape of which varies considerably. It is characteristic in the cat, having been found in every adult examined. It occurs in two forms, either as a bridge connecting the caput and cauda, or as an arm or spur, that is an outgrowth from the caput or cauda, but not joining the two limbs. The former type is by far the most frequent. Of 35 cases examined, the bridge type was presented in 25, the arm or spur type in four, an incomplete formation in five, while in one case, a young animal, it was lacking altogether.

Gage, 78, Schirmer, 93, and Heuer, 06, have shown that both ducts normally exist in the adult, and have the same relation as found in the embryo,—the duct of the dorsal pancreas enters the intestine beyond the orifice of the common bile duct.

HUMAN EMBRYOS.

The pancreas was first studied in man. Wirsung, 1642-43, is considered the discoverer of the duct which opens into the intestine by way of the bile duct.

Vesling, 1664, De Graaf, 1671, and several other investigators of that time, noted the occasional presence of a second pancreatic duct which they considered abnormal.

Santorini, 1775, and Bernard, 56, believed in the normal presence of two pancreatic ducts.

In 85, His published reconstructions of human embryos, showing the dorsal but not the ventral pancreas.

Phisalix, 88, was the first to explain embryologically the presence of two pancreatic ducts. In a human embryo of 10 mm., he found that the pancreas developed from two separate parts. One part corresponded with the "conduit accessoire," and developed from the duodenum a little above the common bile duct. The other, of smaller dimensions, corresponded with the "canal de Wirsung," and was in intimate relations with the bile duct.

Zimmermann, 89, investigated a human embryo of 7 mm., and described a double ventral pancreas, arising from the bile duct.

Felix, 92, figured the pancreas in a human embryo of 8 mm. (Fig. 18, Taf. XVII). He pictures a dorsal pancreas arising from the intestine anterior to the bile duct. The ventral pancreas is double, the left part being rudimentary.

Hamburger, 92, figures models of the pancreas in human embryos of five and six weeks (Fig. 2 u. 3). In both, the duct of the dorsal pancreas enters the intestine nearer the stomach than the bile duct. Hamburger found, in an embryo of four weeks, that the ventral pancreas arose from the intestinal wall some distance below the bile duct. He concluded, therefore, that it secondarily became connected with the latter.

Janošik, 95, represents in Figs. 18 and 20 the pancreas in human embryos of 1 and 2.9 cm. In the younger embryo the opening of the common bile duct is nearer the stomach than that of the dorsal pancreas. In the older embryo the relation is reversed.

Jankelowitz, 95, studied the pancreas in a human embryo of 4.9 mm., and describes a dorsal and a ventral pancreas, the latter composed of right and left divisions.

Swæen, 97, described the pancreas as found in embryos of 10 mm., 18 mm., 15 mm. (nuchal length), and 4.5 cm. His model of the pancreas

of the 10-mm. embryo (Figs. XI and XII, Pl. I) agrees essentially with those of Hamburger. The dorsal pancreas at this stage was separate from the ventral, but in the 18-mm. embryo anastomosis between the two had occurred ventral to the portal vein.

Helly, **01**, figures the pancreas of a 11-mm. human embryo (Fig. 30, Taf. XVII). He represents the dorsal pancreas as arising from the intestine nearer the pylorus than the opening of the bile duct. He also represents a right and a left ventral pancreas arising from the common bile duct. The right ventral pancreas is the larger; the left shows evidence of degeneration. In a later publication, **04**, he cites two cases

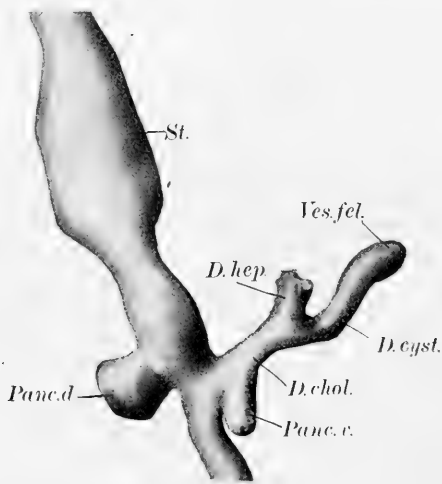


FIG. 5. Reconstruction from a human embryo of 7.5 mm. (H. E. C. 256). $\times 55$ diams. *D. chol.*, ductus choledochus. *D. cyst.*, ductus cysticus. *D. hep.*, ductus hepaticus. *Panc. d.*, pancreas dorsale. *Panc. v.*, pancreas ventrale. *St.*, stomach. *Ves. fel.*, vesica fellea.

(human embryos of 6.5 and 9.5 mm.) where the dorsal pancreas has a pronounced cranial position. Helly believes that in man the final position of the opening of both pancreatic ducts is established at the very beginning of their development.

Völker, **02**, describes two human embryos, one of 3 mm., the other of 13 mm. In the younger embryo the dorsal pancreas is an outpocketing from the dorsal wall of the duodenum. The greater part of it is posterior to the liver. In the older embryo (13 mm.) the duct of the dorsal pancreas opens into the dorsal side of the intestine at about the same level as the common bile duct. In this paper and in a later publication, **03**,

Völker maintains that the bile and pancreatic ducts are not developed at first in their final relation to one another, but that in the course of embryonic development they move in opposite directions, and that in this way their primary relations may be reversed.

Kollmann, 07, shows three original pictures of the pancreas as found in human embryos of 7.5 mm., 5 and 6 weeks (Fig. 394, 395, u. 397). In each of the three embryos the duct of the dorsal pancreas, "ductus pancreaticus secundarius," enters the intestine nearer the stomach than the common bile duct.

Ingalls, 07, figures a model of a pancreas in a 4.9 mm. human embryo (Fig. 3 u. 4, Taf. XXX). He describes a dorsal pancreas and agrees

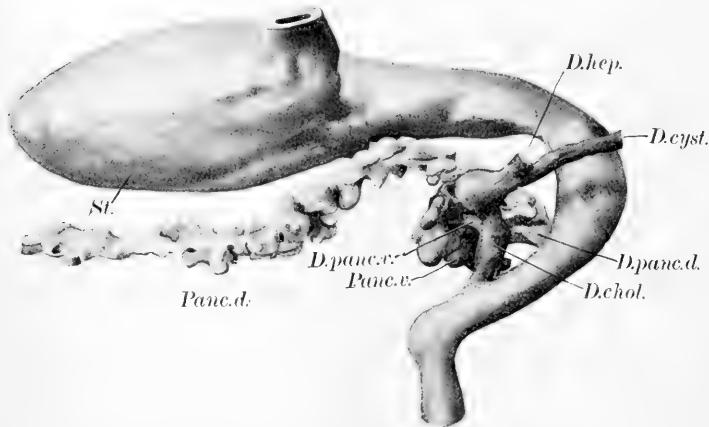


FIG. 6. Reconstruction from a human embryo of 13.6 mm. (H. E. C. 839). $\times 55$ diams. *D. chol.*, ductus choledochus. *D. cyst.*, ductus cysticus. *D. hep.*, ductus hepaticus. *D. panc. d.*, ductus pancreatis dorsalis. *D. panc. v.*, ductus pancreatis ventralis. *Panc. d.*, pancreas dorsale. *Panc. v.*, pancreas ventrale. *St.*, stomach.

with Jankelowitz that the ventral pancreas shows its double origin, "yet there is only a suggestion of the paired condition."

Models of the human pancreas are shown in Figs. 5 and 6. Fig. 5 is from a human embryo of 7.5 mm. (H. E. C. 256). The dorsal pancreas (*Panc. d.*) arises from the dorsal wall of the duodenum a little nearer the stomach (*St.*) than the bile duct (*D. chol.*), the posterior border of the pancreas being approximately on a level with the anterior wall of the bile duct. This pancreatic outgrowth extends into the dorsal mesentery.

A little posterior to the duct of the dorsal pancreas, the common bile duct (*D. chol.*) opens into the ventral side of the intestine. It termi-

nates distally in the hepatic and cystic ducts (*D. hep.* and *D. cyst.*), the latter passing to the ventrally placed gall bladder (*Ves. fel.*). The ventral pancreas (*Panc. v.*) is a small solid mass of cells arising from the posterior wall of the common bile duct, close to its union with the intestine. It extends posteriorly and a little to the right, but is still considerably removed from the ventral border of the portal vein.

The second model (Fig. 6) is from an embryo of 13.6 mm. (H. E. C. 839). It is evident that this is a much more advanced condition than that just described. The model is here represented, somewhat ventrally, from the right side. The stomach (*St.*) has so revolved that its primitive dorsal border or greater curvature is now toward the left, and its ventral border or lesser curvature toward the right.

The dorsal pancreas (*Panc. d.*) is very large as compared with the ventral pancreas (*Panc. v.*). Its duct (*D. panc. d.*) opens into the duodenum nearer the stomach than the bile duct (*D. chol.*), as was shown in the younger embryo. Distally it extends into the mesogastrium. There is, however, no process encircling the right side of the portal vein such as was found in the pig and rabbit embryos, the condition being more like that in the cat of 10.7 mm.

The ventral pancreas (*Panc. v.*), which in the drawing is more darkly shaded than the dorsal pancreas, forms only a very small part of the pancreatic tissue. By the growth of the duodenum the ventral pancreas has been carried into close relation with the proximal part of the dorsal pancreas as happened in the pig embryo (Fig. 2). The two parts of the pancreas (*Panc. d.* and *v.*) already anastomose ventral to the portal vein and on the left of the common bile duct.

The duct of the ventral pancreas (*D. panc. v.*) is well developed at this stage, and according to Hamburger, 92, Schirmer, 93, Joubin, 95, Charpy, 98, Helly, 98, Opie, 03, and Baldwin, 07, its absence is exceptional in the human adult. Thus these investigators confirm the earlier opinion of Santorini and Bernard.

The human pancreatic ducts differ from those of other mammals studied in that usually the duct of the dorsal pancreas opens into the intestine nearer the stomach than the common bile duct; in other mammals the duct of the dorsal pancreas is beyond the bile duct. Even in young human embryos the dorsal pancreas is distinctly anterior to the intestinal orifice of the bile duct (Fig. 5; Felix, Fig. 18, Taf. XVII; Helly, Fig. 30, Taf. XVII; Kollmann, Fig. 394; Ingalls, Fig. 3 u. 4, Taf. XXX; etc.). Several cases, however, are recorded both in the embryo (His, 85, Wlassow, 94, Janošík, 95, Völker, 02 and 03, et al.), and in the

adult (Owen, 68, and others) where the duct of the dorsal pancreas opens into the intestine beyond the opening of the common bile duct. These cases, however, must be exceptions to the normal condition. My evidence for this conclusion is derived from a study of these ducts in the following series of eighteen human embryos in the Harvard Embryological Collection: 256 (7.5 mm.), 817 (8 mm.), 529 (9.4 mm.), 1005 (9.4 mm.), 1001 (9.6 mm.), 1000 (10 mm.), 736 (10.2 mm.), 816 (12 mm.), 839 (13.6 mm.), 1003 (14.5 mm.), 1128 (16 mm.), 1129 (18.1 mm.), 819 (19 mm.), 828 (19 mm.), 851 (22 mm.), 871 (22.8 mm.), 181 (23 mm.), and 38 (24 mm.). In every one of these embryos the duct of the dorsal pancreas was found to be nearer the stomach than the intestinal opening of the common bile duct.

SUMMARY.

From the preceding study it is seen that in the pig, rabbit, cat, and man, there is a dorsal and a ventral pancreas. In the eighteen human embryos examined the dorsal pancreas arises from the intestine distinctly anterior to the hepatic diverticulum, and in the human adult its duct is generally found to be nearer the stomach than the orifice of the bile duct. In the pig, rabbit, and cat, the duct of the dorsal pancreas opens into the duodenum beyond the bile duct.

In the rabbit and the pig the dorsal pancreas shows, very early in its development, distinct right and left lobes spreading from a common stem; such lobes were not found in human embryos. The ventral pancreas is often described as arising from independent right and left halves, of which the left very soon disappears. The present study has shown no sufficient reason for subdividing the ventral pancreas into two independent lateral parts.

The dorsal pancreas sends a *ventral process* on the right side of the right vitelline vein, to fuse with the ventral pancreas. This process is well developed in the pig and rabbit. In the cat of 10.7 mm. it is indicated, and in the adult cat it is sometimes present and sometimes absent (Heuer). In the human embryo it did not occur, and in the pancreas of the human adult its existence has apparently not been recorded.

By the development of the duodenal loop and associated with the rotation of the stomach, the dorsal pancreas is brought into contact with the ventral pancreas at a second point. Here, on the ventral side of the portal vein, the two anastomose. In those cases in which the *ventral process* has fused with the ventral pancreas, a ring of pancreatic tissue surround-

ing the portal vein is thus completed. The perivenous ring is absent in man, occasional in the cat, and characteristically present in the rabbit and pig.

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THE REGULAR OCCURRENCE OF INTESTINAL DIVERTICULA IN EMBRYOS OF THE PIG, RABBIT, AND MAN.

BY

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WITH 5 TEXT FIGURES.

While engaged in the study of a human embryo of 13.6 mm., one of the writers (Dr. Thyng) discovered a knob-like outpocketing of the intestinal epithelium, a short distance beyond the pancreas. This was thought to be an accessory pancreas, and was reported at the twenty-first session of the Association of American Anatomists as "a possible posterior pancreas in mammalian embryos." The other writer, five years before, had made reconstructions of a similar structure found in several rabbit embryos. Together we undertook the further investigation of these knobs or pockets with the following results.

PIG EMBRYOS.

The youngest pig embryos examined measured 5.5 mm. In one of these, as shown in the reconstruction Fig. 1, the intestinal epithelium presents a round knob, just below the dorsal pancreas. The knob, *Div.*, is shown in section in Fig. 3 A. It contains no lumen; there are no mitotic figures among its nuclei. The intestine of this embryo showed only a single diverticulum, but in another pig of 5.5 mm. two were found, as shown in Fig. 2.

Three pigs of 6.0 mm. were examined. Two of these (H. E. C. 918 and 919) each present a single round knob with constricted pedicle; the other (H. E. C. 9) has two diverticula. A mitotic figure is found at the neck of one of the knobs and at the base of another. For a given number of cells it appears that mitoses are more frequent in the knobs than in the surface epithelium, yet from these and other specimens it is evident that mitoses are not confined to the diverticula.

In a 7.0-mm. embryo (H. E. C. 11) two diverticula were found. The anterior of these is merely a hemispherical bulging of the epithelium; the posterior has a constricted neck and is closely applied to the endo-

thelium of the vitelline veins, where they anastomose dorsal to the intestine. (Compare with Fig. 2.)

At 7.8 mm. one very small knob was detected in a somewhat overstained preparation (H. E. C. 428). Along the hepatic diverticulum toward the gall bladder there is a well defined pocket containing a lumen. Such pockets, closely resembling certain of the intestinal diverticula, are of common occurrence along the cystic ducts of embryos. Another pig of 7.5 mm. (H. E. C. 29) shows three diverticula. These are all smaller and less definite than those in the younger stages.

An embryo of 9.0 mm. (H. E. C. 52) shows a small anterior bud, and a large posterior one extending through five 10- μ sections. The latter

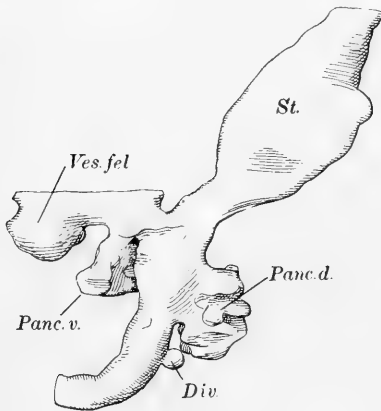


FIG. 1.

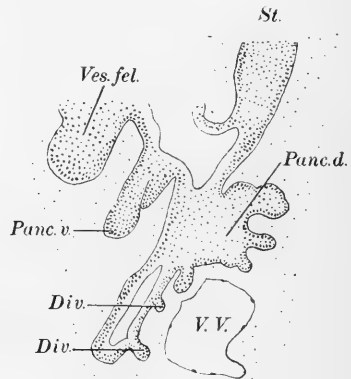


FIG. 2.

FIG. 1. Reconstruction from a pig embryo of 5.5 mm. (H. E. C. 915). $\times 55$ diams.

FIG. 2. Sagittal section (96) from a pig embryo of 5.5 mm. (H. E. C. 916). $\times 55$ diams. *Div.*, diverticulum. *Panc. d.*, Pancreas dorsale. *Panc. v.*, Pancreas ventrale. *St.*, Stomach. *Ves. fel.*, vesica fellea. *V. V.*, vitelline veins.

is a pyriform structure, with the suggestion of an eccentric lumen in the last section; its connection with the intestine occurs in the first two sections. It contains four mitotic figures.

Two embryos of 10.0 mm. were examined, both being rather heavily stained. One showed a single very small knob (H. E. C. 414); in the other no diverticulum could be found (H. E. C. 402).

In a 12-mm. pig (H. E. C. 5) there is a bud, not very well defined, anterior to the dorsal pancreas. Posterior to the pancreas there is a detached nodule of epithelium seen in three sections. It contains a

mitotic figure. The nodule is ventral to the vitelline vein and about equidistant from the pancreatic tubules and the intestinal epithelium. Histologically it has a greater resemblance to the intestine. Another 12 mm. embryo (H. E. C. 518) shows a single small knob, bridging a clear space due to shrinkage between the epithelium and mesenchyma.

Three 14-mm. embryos were examined. Series 65 shows three small buds, and a detached cord of cells seen in seven sections. The cord is found between the superior mesenteric artery and the portal vein. One of the buds is anterior to it; the other two are posterior, being found along the proximal part of the anterior limb of the intestinal loop.

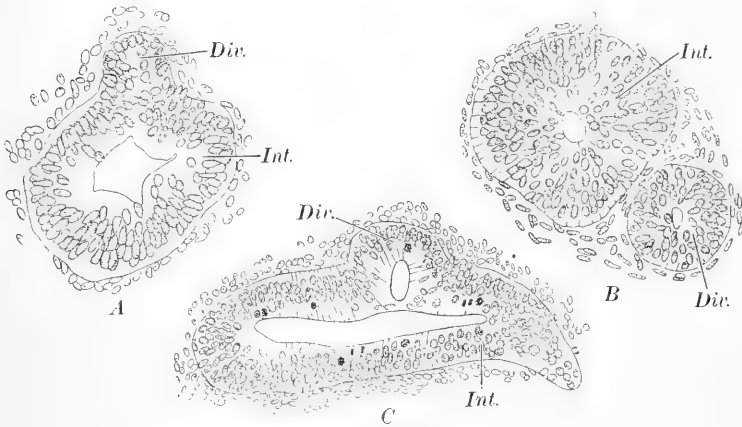


FIG. 3. A, section through the intestine, *Int.*, and diverticulum, *Div.*, in a pig embryo of 5.5 mm. (H. E. C. 915, section 232). $\times 225$ diam. B, similar section from a human embryo of 13.6 mm. (H. E. C. 839, section 527). $\times 225$ diams. The diverticulum connects with the intestine in adjacent sections; compare with Fig. 5. C, similar section from a rabbit embryo of 14 days, 11 mm. (H. E. C. 1327, section 508). $\times 150$ diams.

Within the umbilical cord a part of the intestine has been cut off. Series 66, also a 14-mm. embryo, shows in addition to a small knob, two elongated pyriform diverticula. The first of these is seen in five $20\text{-}\mu$ sections. It is nearly, but not quite, detached from the intestine. The second is also pyriform, its distal diameter being $30\ \mu$. Neither of these diverticula contains a lumen, yet the nuclei are arranged peripherally, as in a simple epithelium. The small knob occurs beyond these diverticula, on the anterior limb of the intestinal loop. Another 14 mm. embryo (H. E. C. 1130) shows two detached epithelial nodules and one small pearl or bud.

An embryo of 17 mm. (H. E. C. 51) shows nine diverticula, most of which have a distinct lumen communicating with that of the intestine. They begin below the pancreas and are distributed along the duodenal region and anterior limb of the intestinal loop. There are none on the posterior limb; within the umbilical cord a portion of the intestine has been cut away. Another 17-mm. embryo (H. E. C. 50) has ten diverticula and one detached nodule. These are limited to the duodenum and small intestine; a portion of the intestine has been cut away.

A pig of 20 mm. (H. E. C. 59) contains eleven diverticula of the small intestine. A part of the intestine has been cut off with the umbilical cord. In another pig of 20 mm. (H. E. C. 60) there is a hemispherical diverticulum just anterior to the duct of the dorsal pancreas, as shown in Fig. 2 (p. 491) of the preceding article in this journal.¹ Beyond the dorsal pancreas there is an elongated, unbranched diverticulum .55 mm. long and about .07 mm. in diameter, therefore much larger than any of those already described. It is shown in the figure as an "accessory pancreas." As it crosses the muscle layer of the intestine it is greatly constricted, suggesting that it may become detached at this point. Beyond this "accessory pancreas" there are 18 diverticula along the anterior limb of the intestinal loop. In most of these there is a distinct lumen leading into the intestine. Similar diverticula occur along the cystic duct. There are none along the posterior limb of the intestinal loop; a part of the loop within the umbilical cord has been cut away. A third specimen of a 20-mm. embryo (H. E. C. 61) has 12 diverticula in that part of the small intestine which is preserved. There are none along the large intestine. The interesting feature of this embryo is a rather thin-walled epithelial cyst with a few rounded outpocketings, found just outside the muscularis of the duodenum, a short distance from the pancreas (Fig. 4). The intestine near by presents a solid cylindrical outgrowth which extends to the muscularis, but does not penetrate it. Undoubtedly this was formerly connected with the cyst, although at present it is not directed toward it, and is not where the cyst approaches nearest to the muscularis. It appears that after the stalk became detached, the growth of the intestine carried it along and changed its direction. The epithelium of the cyst, which corresponds with the "accessory pancreas" of the last specimen, is quite unlike that of the pancreas. The pancreatic tubules, about 0.05 mm. in diameter, contain only a minute lumen,

¹Thyng, F. W. Models of the pancreas in embryos of the pig, rabbit, cat, and man. *Amer. Journ. of Anat.*, 1908, Vol. 7, p. 489-504.

whereas the cyst has a cavity 0.22 mm. in diameter, equaling that of the intestine.

Two embryos of 24 mm. were examined. In both of these some of the intestine within the umbilical cord has been cut away. One series (H. E. C. 62) contains 14 diverticula and the other (H. E. C. 64) has 15. There are none in the large intestine.

A partial series from a pig of 32 mm. (H. E. C. 136) includes the large intestine to its junction with the ileum. Beyond this point the ileum and caecum continue for a short distance and then are both cut away.

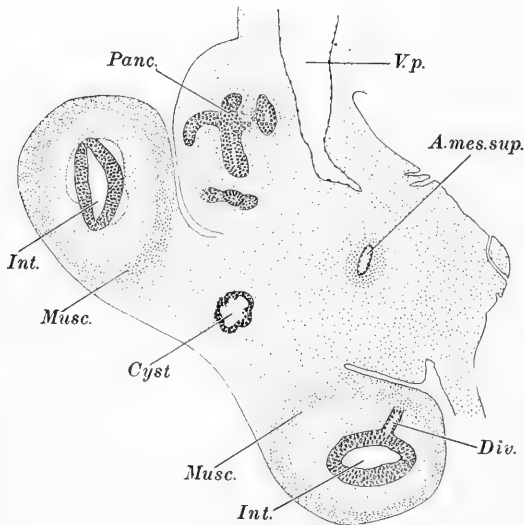


FIG. 4. Section from a pig embryo of 20 mm. (H. E. C. 61, section 228). $\times 50$ diams. The loop of the duodenum is cut in two places, *Int.* Midway between these, the *Cyst* comes in contact with the muscularis, *Musc.*, in the following sections. *Div.*, diverticulum. *Panc.*, pancreas. *A. mes. sup.*, arteria mesenterica superior. *V. p.*, vena portae.

Where the caecum joins the colon there is an epithelial proliferation subdividing the lumen. Here there is a cluster of diverticula, two of which are quite distinct. In the remainder of the colon and in the rectum, diverticula are absent. The pockets of the small intestine, as seen in wax reconstructions, are bean-shaped structures attached to the intestine the whole length of their narrow edge. The connection with the intestine is laterally compressed, but extends longitudinally through several sections. Thus the knobs appear as if modified by the lengthwise growth of the intestine.

The occurrence of diverticula in the 24 pig embryos already described may be tabulated as follows:

Series No.	Length.	Diverticula.	Series No.	Length.	Diverticula.
915	5.5 mm.	1	518	12.0 mm.	1
916	5.5 mm.	2	65	14.0 mm.	3 + 1 detached
918	6.0 mm.	1	66	14.0 mm.	3
919	6.0 mm.	1	1130	14.0 mm.	1 + 2 detached
9	6.0 mm.	2	51	17.0 mm.	9
11	7.0 mm.	2	50	17.0 mm.	10 + 1 detached
428	7.8 mm.	1 very small	59	20.0 mm.	11
29	7.8 mm.	3	60	20.0 mm.	20
52	9.0 mm.	2	61	20.0 mm.	12 + 1 detached
414	10.0 mm.	1 very small	62	24.0 mm.	14
402	10.0 mm.	0	64	24.0 mm.	15
5	12.0 mm.	1 + 1 detached	136	32.0 mm.	present.

Summary.—In pig embryos from 5.5 to 14 mm. in length, one or two knob-like diverticula occur regularly in the duodenal region. Sometimes they are small as in Series 428 and 414, or absent as in Series 402, but usually they are easily found. In embryos from 14 to 24 mm. the number of diverticula increases and they are distributed along the small intestine. None were found in the large intestine, except in the 32-mm. specimen, where a cluster of diverticula occurred near the ileum. The diverticula appear first in the duodenum, and later in the lower portion of the small intestine. They begin as round knobs which may become elongated and detached from the intestine in the form of nodules, strands or cysts. In later stages they acquire a lumen and those found in the distal part of the small intestine appear as flask-shaped gland-like pockets.

RABBIT EMBRYOS.

At 11 and 11½ days, rabbit embryos contain both the dorsal and the ventral pancreas, but no intestinal diverticula. This is true of some at 12 days, but Series 147, of a 5-mm. rabbit of 12 days, shows a pearl-like disturbance of the epithelial cells, suggestive of the later pockets. At 13 days (H. E. C. 153, 7.5 mm.) a round pocket with a lumen emptying into the intestine occurs just beyond the duct of the dorsal pancreas. The pocket is 0.07 mm. in diameter and extends through six sections. In this embryo there are also three pearls along the anterior limb of the intestinal loop.

At 14 days (H. E. C. 156, 10.5 mm.) there is a duodenal pocket, near which a detached epithelial nodule, containing a lumen, appears in three

sections. There are four pearls along the anterior limb of the intestinal loop.

At 16½ days (H. E. C. 575, 18.8 mm.) there are six pockets, each with a lumen. The largest of these, 0.1 mm. in diameter, is in the duodenum. The others are in the coiled part of the small intestine, separated from the first by a considerable interval. They do not decrease regularly in size toward the colon. No pockets or pearls were found in the large intestine.

An embryo of 17 days, 22 mm. (H. E. C. 165), and another of 18 days, 25 mm. (H. E. C. 168) each show a well-defined pocket in the duodenal region and another in the umbilical coils of the intestine, but in each case a considerable portion of the intestine has been cut away.

Two embryos of 20 days, 29 mm., were studied. In Series 170, six pockets were found and modeled in wax. They occur at irregular intervals along the distal half of the small intestine; there is no pocket in the duodenum. The models show that the pockets are nodular bulgings of the epithelium, quite distinct from folds; they vary in diameter from 0.05 to 0.16 mm. The other embryo of the same age and size shows only three pockets. These are in the distal half of the small intestine.

A part of the intestine of a rabbit of 41.6 mm. was examined and a single pocket found. It is shaped like a flat, round flask, and is set in the epithelium so that it produced only a slight bulging of the basement membrane. It has an oval lumen emptying into the intestine, and is lined with smooth epithelium, contrasting sharply with the much folded intestinal layer which is in process of forming villi. The diverticulum is 0.16 mm. in diameter. It shows no evidence of glandular activity and is not surrounded by lymphoid tissue.

The rabbit embryos which have been described indicate that the diverticula begin to develop at 12 days. They have been recorded as follows:

Series No.	Length.	Duodenum.	Small intestine.
147	5.0 mm.	1 pearl	0
153	7.5 mm.	1 pocket	3 pearls
156	10.5 mm.	1 "	4 "
575	18.8 mm.	1 "	5 pockets
165	22.0 mm.	1 "	1+? "
168	25.0 mm.	1 "	1+? "
170	29.0 mm.	0 "	6 "
171	29.0 mm.	0 "	3 "
...	41.6 mm.	? "	1+? "

In addition to the rabbit embryos included in this table, 10 others ranging from 12 to 22 days were examined, all of which showed at least

one pocket. The duodenal diverticulum in one of these is shown in the reconstruction Fig. 3 on p. 494 of the preceding article; a section through it is seen in Fig. 3 C. As in the pig, mitotic figures are not limited to the diverticula.

HUMAN EMBRYOS.

An embryo of 4.0 mm. (H. E. C. 714) has previously been described by Dr. Bremer² as presenting two solid epithelial knobs on the mid-ventral border of the intestinal tube. One of these is just beyond the hepatic diverticulum; the other is nine sections further on, at the place where the intestine begins to expand to form the yolk sac. The second

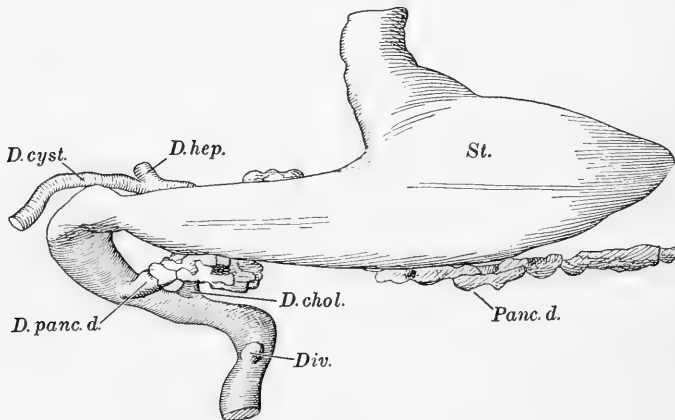


FIG. 5. Reconstruction from a human embryo of 13.6 mm. (H. E. C. 839). $\times 55$ diams. *Div.*, diverticulum. *D. chol.*, ductus choledochus. *D. cyst.*, ductus cysticus. *D. hep.*, ductus hepaticus. *D. panc. d.*, ductus pancreatis dorsalis. *Panc. d.*, pancreas dorsale. *St.*, stomach.

knob, which is seen in two sections, is in contact with the intestinal epithelium, but is, apparently detached. The dorsal pancreas has not appeared.

In five much older embryos, measuring from 7.5 to 10.2 mm., no diverticula were found. In an embryo which in alcohol measured 13.6 mm. (H. E. C. 839), there is a well developed duodenal pocket shown in the reconstruction (Fig. 5) and in the section (Fig. 3 B). Above this diverticulum the lumen of the duodenum is subdivided into two or three parts by the proliferation of the epithelium. Such proliferation, which

² Bremer, J. L. Description of a 4-mm. human embryo. *Amer. Journ. of Anat.*, 1906, Vol. 5, p. 459-480.

has been studied by Tandler³ is independent of the formation of the diverticula. Below the duodenal pocket, indications of diverticula may be observed at twelve places along the small intestine. There are transitions between well defined diverticula and slight irregularities of the epithelium which may be without significance.

Series 181, an embryo of 23.0 mm., shows well-developed pockets. Several of the sections are misplaced and the counting of the diverticula is somewhat difficult, but there appear to be 33 along the small intestine. There are none in connection with the vermiform process or large intestine.

An embryo measuring 22.0 mm. (H. E. C. 851) is obviously more advanced than the preceding embryo of 23.0 mm.; its shorter length is due to greater curvature of the back and flexion of the head. The epithelium in the proximal part of the small intestine is greatly folded and has shrunken from the mesenchyma, so that diverticula would be difficult to recognize. Within the umbilical cord the intestine is well preserved and presents 48 pockets, generally flask-shaped. Each has a lumen connecting with that of the intestine. There is a well-developed pocket 17 $12\text{-}\mu$ sections above the large intestine, and a slight one only six sections above it, but throughout the large intestine and vermiform process no pocket occurs. The lining of the large intestine is generally smooth, except in the rectal region; that of the small intestine is considerably folded.

In the largest series available (H. E. C. 292), from an embryo of 32 mm., the large intestine was carefully examined. The rectum showed highly developed folds among which a pocket might be hard to detect. None was found. The upper portion of the large intestine showed usually a smooth reniform or trifoliate lumen, and was without a single pocket. There were none in the vermiform process, but they occurred in the ileum near its termination.

The preceding study of human embryos shows that solid knob-like diverticula of the intestine may occur before the formation of the dorsal pancreas, but that older embryos, from 7.5 to 10.0 mm., may be without them. An embryo of 13.6 mm. shows one prominent duodenal pocket, with indications of 12 others along the small intestine. At 23 mm., 33 pockets were counted, and in an older embryo there were 48. In these, and in an embryo of 32 mm., no diverticula were found along the large intestine and vermiform process.

³ Tandler, J. Zur Entwicklungsgeschichte des menschlichen Duodenum. Morph. Jahrb., 1900, Vol. 29, p. 187-216.

SIGNIFICANCE OF THE DIVERTICULA.

Völker⁴ has interpreted a slightly elongated diverticulum in a pig embryo as an accessory pancreas. The length of the embryo is not stated, but the reconstruction of it agrees closely with Fig. 1. Völker describes it as follows:

In this embryo I have made a very interesting discovery, namely that a new accessory pancreas arises from the lumen of the intestine almost in the mid-ventral line and in about the third section beyond the connection of the pancreas with the intestine; it can be followed distally for about four sections. It consists of polyhedral cells of exactly the same character as those in the rest of the pancreas, but it has no lumen. In spite of the most careful investigation I could not demonstrate a similar structure in any other embryo. It is probably to be regarded as an individual variation.

Thyng, in the preceding article in this journal, likewise interprets an elongated diverticulum in the pig embryo of 20 mm. as an accessory pancreas. Bremer regards the mid-ventral epithelial knobs in the 4 mm. human embryo as pancreatic, although anomalous in position and time of development.⁵

The conditions in the lower vertebrates do not account for a series of pancreatic outgrowths. Cyclostomes and selachians are said to have only a dorsal pancreas; teleosts, amphibians and higher groups have one dorsal and one ventral pancreas, the lateral lobes of the latter often being counted as two. In the sturgeon, however, von Kupffer⁶ has described and figured a third outgrowth—a median dorsal structure posterior to the others. A portion of it is thought to give rise to the spleen, but the remainder fuses with the other two outgrowths to make the pancreas of the adult. Stöhr⁷ has denied the correctness of these observations, and, although reaffirmed by von Kupffer,⁸ they lack confirmation.

It is well known that a small pancreas may develop at various places along the human small intestine. Through the kindness of Dr. James H. Wright we have examined sections from four such cases—one from

⁴ Völker, O. Beiträge zur Entwicklung des Pankreas bei den Amnioten. Arch. f. mikr. Anat., 1902, Vol. 59, p. 62-93.

⁵ Bremer, J. L. Description of a 4-mm. human embryo. Amer. Journ. of Anat., 1906, Vol. 5, p. 474.

⁶ von Kupffer, C. Ueber die Entwicklung von Milz und Pankreas. Münchener med. Abh., 1892, VII Reihe, 4 Heft, 17 pp.

⁷ Stöhr, P. Ueber Entwicklung von Hypochorda und Pankreas bei Rana. Verh. d. anat. Gesellsch., 9th Versammlung, 1895, p. 176-179.

⁸ See the discussion of Professor Stöhr's paper to which reference has just been made.

the duodenum, two from the jejunum, and one from the umbilicus.⁹ The position of these structures accords with that of the early diverticula. The fact that an accessory pancreas with characteristic islands has been known to occur at the yolk stalk is in favor of Bremer's interpretation of the nodular diverticulum. Opie¹⁰ records the presence of two accessory pancreatic glands in a single individual.

In describing the hepatic diverticulum of a 12-mm. pig it has been stated that "sometimes a knob-like bud is found on its surface. Nearer the intestine these buds are more numerous. Some of them terminate in hepatic cylinders; others end blindly or are found as detached cysts in the liver."¹¹ These structures are presumably the ones which Laguesse¹² described in sheep embryos as pancreatic. He says:

In the sheep I have found a whole series of small new buds arising late from the epithelium of the ductus choledochus and forming as many accessory pancreatic glands In an embryo of 13 mm. none had appeared. None at 48 mm. At 65 mm., on the contrary, numerous little buds arose from the epithelial wall all along the duct but particularly near its outlet. Most of them appeared as tortuous tubes, some having branches. In an embryo of 82 mm. a part of the tubular diverticula ended in simple or lobed secretory cavities, already presenting centro-acinal cells and chief cells containing refractive droplets like zymogen. I feel justified therefore in considering them to be true small pancreatic glands.

We have not had an opportunity to examine such large embryos as Laguesse used, but in a sheep of 24.1 mm. (which shows several intestinal pockets) there are a few outpocketings along the bile and cystic ducts. One of these, somewhat elongated, is near the duct of the ventral pancreas. It is possible that the distal pockets may give rise to diverticula of the gall bladder, such as are found occasionally in the adult.¹³ That the others are pancreatic rather than aberrant hepatic structures remains to be established.

⁹ Wright, J. H. Aberrant pancreas in the region of the umbilicus. *Journ. of the Boston Soc. of Med. Sci.*, 1901, Vol. 5, p. 497-498.

¹⁰ Opie, E. L. Anatomy of the pancreas. *Johns Hopkins Hospital Bulletin*, 1903, Vol. 14, No. 150, p. 229-232.

¹¹ Lewis, F. T. The gross anatomy of a 12-mm. pig. *Amer. Journ. of Anat.*, 1903, Vol. 2, p. 217.

¹² Laguesse, E. Sur l'existence de nouveaux bourgeons pancréatiques accessoires tardifs. *Comptes rendus de la Soc. de Biol., Paris*, 1895. Ser. 10, Vol. 2, p. 602-603.

¹³ Weltz, H. Über Divertikel der Gallenblase. *Inaug.-Diss.*, Kiel, 1894. 20 pp.

It will be difficult to prove that any of the intestinal diverticula are pancreatic. Even the long unbranched diverticulum extending into the territory of the pancreas in the 20-mm. pig embryo might remain in that condition throughout life. Roth¹⁴ describes a diverticulum of the duodenum in the adult, situated 3 cm. from the outlet of the common bile duct. It is 1.5 cm. deep, and consists of a mucosa and a thin submucosa; the muscularis encircles it near its outlet. Its distal part is completely surrounded by pancreatic tissue. He reports four other cases of duodenal diverticula, in two of which the outpocketings extend into the pancreas. In one case the diverticulum had rounded subdivisions suggesting branches. Roth records two, other writers as many as four, diverticula in a single duodenum.

Similar diverticula are well known to occur in any part of the intestinal canal, often in large numbers. Osler¹⁵ reports three cases as follows:

In one of the cases the jejunum presented 53 diverticula on the mesenteric border, all of hemispherical shape and attached by broad bases. They ranged in size from a cherry to a large apple There were not any in the ileum or colon.

In the large intestine I have met with two instances of curious diverticula forming globular sacculi the size of large peas or cherries; very numerous in one case along the whole colon, in the other confined to the lower part.

Such diverticula are commonly ascribed to constipation, marasmus, etc., rather than to developmental factors. They differ from the pockets which we have found in the intestine of the human embryo in their greater size and larger number, in their occurrence only along the mesenteric attachment, and in their distribution which includes the large intestine. In older embryos than those examined it is possible that pockets may be found in the large intestine, especially since there is a tendency for them to develop down the intestinal tract, appearing first near the duodenum. It is possible, also, that they may become stretched out and obliterated along the convex surface of the intestinal loops, or may become directed toward the mesentery during the growth of the intestine, thus giving rise to a mesenteric distribution. If the diverticula of the embryo produce those of the adult, it is through pathological development and distension; their normal development is therefore to be sought.

¹⁴Roth, M. Ueber Divertikelbildung am Duodenum. Arch. f. path. Anat. u. Phys., 1872, Vol. 56, p. 197-201.

¹⁵Osler, W. Notes on intestinal diverticula. Annals of Anat. and Surg., 1881, Vol. 4, No. 5.

Stöhr¹⁶ has found in the small intestine of guinea-pigs eight days old, "primary submucous glands" which penetrate the muscularis mucosae and expand in the submucosa. They are surrounded by ordinary connective tissue. In older guinea-pigs all such glands are in relation with lymph nodules. At the beginning of the colon there is a lymphoid structure called the "tonsilla colica." Here the primary submucous glands give off secondary buds from their expanded basal portion as described by Stöhr in guinea-pigs, and also by Czermack, who notes that the lateral secondary pockets are rudimentary in the rabbit.

Whether the lymphoid cells which invest these pockets arise from the epithelium, as believed by Retterer, von Davidoff, and others, or from the surrounding tissue, as described by Stöhr, Czermack, and others, need not be discussed here. It is agreed that the lymphoid cells appear, and that the pockets become relatively small. The round lumen may give place to a cleft parallel with the intestinal surface, as figured by von Davidoff¹⁷ from a section of the vermiform process of an adult guinea-pig. Czermack¹⁸ states that the epithelium may be partly transformed into bodies comparable with the thymic corpuscles.

In the pig there are lymph nodules on the valve of the colon which Klein¹⁹ describes as follows:

Each of the nodules surrounds a group of Lieberkühn's crypts which—different from the ordinary type—*extend with their fundus through the muscularis mucosae into the submucous tissue.* This group of Lieberkühn's crypts generally opens through a longer or shorter common duct on the surface. The most typical arrangement is this: a group of 10 to 20 small Lieberkühn's crypts situated in the submucous tissues and surrounded by a lymph follicle opens into a common cavity from which a duct passes through the muscularis mucosae to the free surface. I have referred to these glands as "flask-shaped glands."

The extension of intestinal glands (crypts of Lieberkühn) through the muscularis mucosae in relation with a lymph nodule is a common occur-

¹⁶ Stöhr, P. Ueber die Entwicklung der Darmlymphknötchen und über die Rückbildung von Darmdrüsen. Arch. f. mikr. Anat., 1898, Vol. 51, p. 1-55.

¹⁷ von Davidoff. Untersuchungen über die Beziehungen des Darmepithels zum lymphoiden Gewebe. Arch. f. mikr. Anat., 1887, Vol. 29, p. 495.

¹⁸ Czermack, N. Einige Ergebnisse über die Entwicklung, Zusammensetzung und Function der Lymphknötchen der Darmwand. Arch. f. mikr. Anat., 1893, Vol. 42, p. 581-632.

¹⁹ Klein, E. Report on infectious pneumo-enteritis of the pig. 7th Ann. Rep. of the Local Government Board. Supplement for 1877, appendix B. London, 1878, p. 169-280.

rence. Retterer²⁰ has figured such cases, and one of Klein's figures suggests that an area of intestinal glands has become depressed to form a pocket. Such formations have little resemblance to the intestinal diverticula. Several of Klein's figures, however, show the same sort of submucous glands as Stöhr found in the guinea-pig, and these strikingly resemble the diverticula. In the pig after birth, Klein found them only in the large intestine.

We are indebted to Professor Theobald Smith for a demonstration of these structures in a young pig. Their relation to certain infections, as described by Klein, is important, for they become centers of ulceration. Often their lumen contains a concretion which is easily macroscopic.

In discussing Retterer's paper, to which reference has been made, His pointed out that lymphoid tissue develops in relation with very various epithelial folds and pockets, such as the vermiform process, the tonsillar pits, pharyngeal recess, and intestinal glands. If this "general principle" is true, then the relation of the flask-shaped pockets of the intestine to lymphoid tissue is secondary, and the function of the pockets themselves must still be sought.

Stöhr (l. c., p. 24) states that the structures in question are "diverticula like the other intestinal glands, and are distinguished from them only by their breadth and the possession of lateral sprouts. The name glands is indeed unfortunate since they produce no specific secretion." Like the intestinal glands, they may produce mucus, but this is not, in Stöhr's opinion, a sufficient reason to call them glands rather than pits. Klein speaks of their distension with mucus.

Solid knob-like diverticula similar to those of mammalian embryos have been found by Bizzozero²¹ along the intestine of the tailed amphibia. They contain four elements—young epithelial cells, young mucous cells, cells in mitosis, and coarsely granular leucocytes. Bizzozero places emphasis upon the mitoses, and regards the knobs as centers for the production of cells to replace those lost from the surface. The knobs are less definite in the tail-less amphibia, in the lower vertebrates, and in reptiles. In mammals the mitotic centers are near the bases of the intestinal glands, and Bizzozero considers that these glands are therefore comparable with the solid diverticula of the amphibia. In various insects

²⁰ Retterer. Sur l'origine des follicules clos du tube digestif. *Verh. d. anat. Gesellsch.*, 9th Versammlung, 1895, p. 31-39.

²¹ Bizzozero, G. Ueber die Schlauchförmigen Drüsen des Magendarmkanals und die Beziehung ihres Epithels zu dem Oberflächenepithel der Schleimhaut. *III. Arch. f. mikr. Anat.*, 1893, Vol. 42, p. 82-152.

he has found diverticula, sometimes solid and sometimes glandular, which are regenerative centers. He states that in *Melolontha vulgaris* "we find structures such as we have learned to recognize in Triton."

Nicolas²² has verified the occurrence of knobs along the intestinal epithelium of Salamandra. He found mitotic figures and the three sorts of cells described by Bizzozero, and states that the buds occur over the whole extent of the small intestine and rectum. He disagrees with Struiken, who has said that these structures are not found in the rectum of the salamander, but admits that there they are much less developed and approach the larval form. He believes that during the winter, when intestinal activity is at a minimum, the buds are relatively rare.

CONCLUSIONS.

Knob-like intestinal diverticula occur regularly in embryos of the pig, rabbit, and man. Their presence is noted also in the cat and sheep.

Similar diverticula are found along the cystic and bile ducts. These may give rise to glandular tissue (hepatic or pancreatic); they may degenerate and disappear, sometimes after becoming detached in the form of epithelial nodules or cysts; or they may possibly form permanent diverticula of the gall bladder.

The knob-like intestinal diverticula are the probable source of an occasional accessory pancreas. They usually degenerate, sometimes forming detached cysts and nodules.

The diverticula are more numerous in the older embryos studied, and occur especially in the distal part of the small intestine. Although none were found in the large intestine, except near the valve of the colon in the oldest pig examined, it is possible that they may appear there in later stages.

The distal diverticula probably correspond with the "flask-shaped glands" (Klein) or "primary submucous glands" (Stöhr), which become surrounded by lymphoid tissue and nodules. In this they resemble various other epithelial pockets.

It is possible that these structures may give rise to pathological "diverticula."

The diverticula, although they are not exclusive centers of cell division, are comparable with the knob-like proliferations of the intestinal epithelium in the tailed amphibia, as described by Bizzozero and Nicolas.

²²Nicolas, A. Les bourgeons germinatifs dans l'intestin de la larve de salamandre. *Bibliogr. Anat.*, 1894, Vol. II, p. 37-42.

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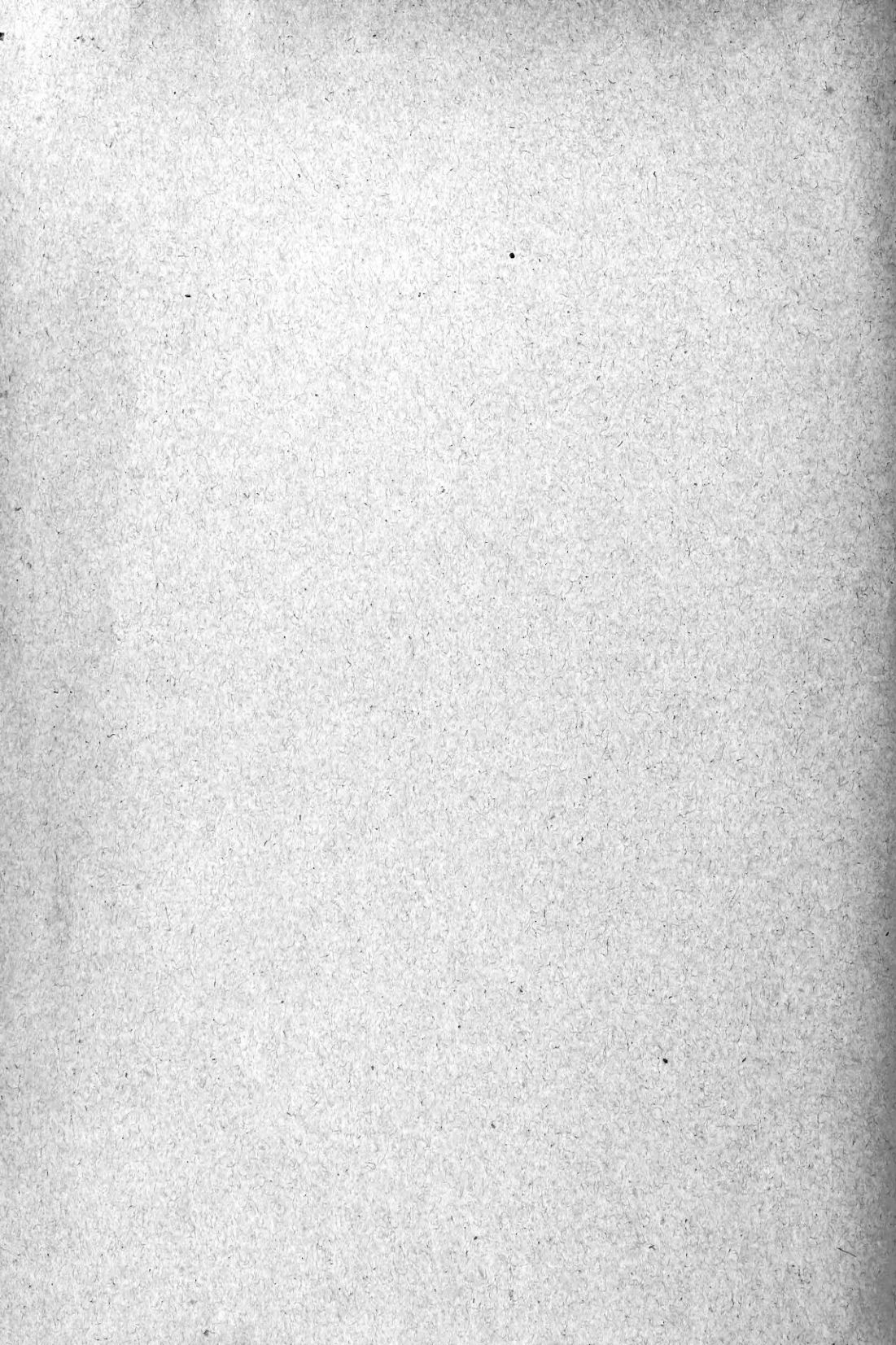
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